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Detection and Molecular Characterization of Phytoplasma and its Effect on the Vitamin and Fibers in *Corchorus olitorius* Crops in Egypt

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A PHYTOPLASMA was detected in the *Corchorus olitorius* (Mallow) crop in 2022 at Fayoum governorate, Egypt. Infected *C. olitorius* showed symptoms of phyllody, leaf deformations, and slight yellowing. Total DNA was extracted from symptomatic or asymptomatic leaf tissues and was tested for phytoplasma presence using nested polymerase chain reaction (PCR). A fragment was amplified from symptomatic samples using universal phytoplasma primer set P1/P7 and primer set R16F2n/R16R2. The amplicon for these primers is 1271 bp, but the DNA of asymptomatic samples did not yield an amplicon. The nested PCR products were sequenced with R16F2n/R16R2 primers, and then sequences were aligned through the DNA-man program V7. Based on the phytoplasma found in this study (Acc. No. OQ836595), the results imply 98.1% sequence identity with the phytoplasma of *C. olitorius* (OP889144) from Egypt, China (OP889144 and KM103730), and from other Indian crops. Identifying the vector species that is spreading the discovered phytoplasma in the Mallow crop is still necessary. Many active phytochemicals found in *C. olitorius* plants are of great interest due to their potential to improve human health. This study demonstrates that in plant leaves, phytoplasma presence lowered the tocopherol (Vitamin E) content and increased the crude fiber content.

Keywords: Chemical analysis, Molecular techniques, Phyllody dis

Introduction

The mallow (molokhia) plants (*Corchorus olitorius* L.), also known as Jew's mallow, wild okra, jute mallow, or tossa jute, are primarily herbaceous perennials from edible leafy vegetables or fiber crop in the Malvaceae family and present naturally in the Mediterranean (Cagirgan et al., 2014; Abdel-Razek et al., 2022). With a 2.45tons/ha productivity, Egypt's 887 hectares of jute mallow farming produced about 2173 tonnes of output (Haridy et al., 2019). Studies have shown that this plant plays a relevant role in maintaining human health, and the plant possesses a wide range of biological activities connected to its phytochemical composition (Moerman, 1996; Hussien et al., 2017; Abdel-Razek et al., 2022) as the leaves of *C. olitorius* include minerals, vitamins, and phenolic compounds that are abundant in plants (Schipper, 2000; Abdel-Razek et al., 2022). The leaves are used in herbal pharmacopeia (Adebo et al., 2018) to treat gonorrhea, pain, fever, tumors, an emollient, diuretic, and dysentery (Ndlovu & Afolayan, 2008; Abdel-Razek et al., 2022) and preventing the risk of Alzheimer's disease (Mangione et al., 2022) or as a potential substitute for the generation of biofuel (Cagirgan et al., 2014).

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Prokaryotic plant pathogens known as phytoplasmas were first identified in 1967 by Doi et al. (Doi et al., 1967). Because phytoplasmas are difficult to cultivate in artificial media (Contaldo & Bertaccini, 2019), the methods used for bacterial identification are not applicable to these organisms. To accurately identify phytoplasma infections in *C. olitorius* plants, nested PCR tests targeting the 16S rRNA gene region of the pathogen were conducted after polymerase chain reaction (PCR) tests. Phytoplasma diseases impact many plant species, including mallow plants (Biswas et al., 2014; Inaba et al., 2022). According to Bertaccini & Duduk (2009), Hogenhout & Segura (2010), and Mokbel & Kheder (2020) phytoplasma-symptoms include the transformation of flowers into leafy structures, the greening of flower tissue (virescence), a proliferation of shoots that resemble witches' brooms, and changes in the color of leaves or shoots. The first reports of phyllody disease in *C. olitorius* plants were made by Cagirgan et al. (2014) in Turkey. Also, Biswas et al. (2014) discovered that *C. olitorius* plants have phytoplasmas associated with symptoms like small leaves and bunchy tops. Jute plants were identified by Ozdemir & Cagirgan (2015) as weeds in experimental sesame trials carried out at the Akdeniz University campus in Antalya. Weed characteristics included abnormally formed flowers, smaller leaves with short internodes, and yellowing. Additionally, it has been demonstrated that plants with phytoplasma infection might act as a pathogen reservoir (Borroto-Fernandez et al., 2007).

The goals of this research were to: (1) Use molecular techniques to detect and characterize the phytoplasma from infected *C. olitorius* plants in Egypt; (2) Determine the leaf vitamin content; and (3) The crude fiber content in healthy and phytoplasma infected plants.

Materials and Methods

Plant sampling

In 2022, leaf deformations and phyllody symptoms with tenuous yellowing on plant leaves (Fig. 1) were observed in *C.olitorius* plants grown in a Tamyia region (Kafr Mahfouz) inside the Fayoum Governorate. To detect and identify plant pathogens and their impact on the phytochemicals of the leaves, symptomatic and asymptomatic plants were tested.

Nucleic acid extraction and nested PCR detection

Total DNA was extracted from symptomatic and asymptomatic leaf samples as well as healthy ones as described by Dellaporta et al. (1983). The primers P1/P7 and R16F2n/R2 (Deng & Hiruki, 1991; Gundersen & Lee, 1996) were used for nested PCR. The PCR products were electrophoresed on a 1% agarose gel, stained with EZView St Postivain (Biomatik - Canada), and then observed under a UV light source.

Sequencing

Amplified PCR products were purified using a PCR purification Kit (QIAquick®) according to the manufacturer's instructions. The Macrogen Company (South Korea) performed direct sequencing on the purified PCR product. The Gen Bank received the nucleotide sequence submissions. Using DNA-Man program V7, the arrangements were examined and compared with other sequences that were accessible in GenBank.

Chemical analysis of leaves of Corchorus olitorius plants

The chemical analysis was carried out on the powdered three samples of *C. olitorius* plants by Food Safety and Quality Control Laboratory (Cairo University) using a spectrophotometer and HPLC. All chemicals, solvents, and the standards used in this study were of analytical grade (Sigma Aldrich). Asymptomatic leaf samples of three different *C. olitorius* plants were used for comparison of phytoplasma effects on plants. Water soluble vitamin in both healthy and infected ones with phytoplasma was determined using HPLC (Agilent, USA), equipped with a Quaternary pump, and a HyperClone TM BDS C18, 130A 100mm x 4.6mm (Phenomenex, USA) and operated at 35C. The separation was achieved using a binary linear elution gradient with (A) HPLC grade water 0.1% Formic acid (v/v), and (B) Acetonitrile. The Injection volume was 20µL and detected with a VWD detector at 245nm based on the procedures followed by the Analytical Methods Committee (1959). The effect of infection with phytoplasma was quantified using a UV spectrophotometer (Jenway, England) using the Weende method for determining crude fiber in plants (AOAC, 2005) by acid hydrolysis with 1.25% H2SO4 that was used for the extraction processes, followed by alkaline hydrolysis with 1.25% NaOH.

Results and Discussion

C. olitorius plants in a Kafr Mahfouz region inside the Fayoum governorate in Egypt, which showed typical symptoms of phytoplasma disease, were observed in 2022. Symptoms consisted of crinkled and slightly yellow leaves, abnormally developed leafy structures, and phyllody (Fig. 1A-C) as compared to healthy plants (Fig. 1D).

Currently, phytoplasmas are among the most dangerous plant diseases that harm economically essential crops (Bertaccini, 2021; Ahmed et al., 2021; Hemmati et al., 2021). Despite being a sign of the phytoplasma disease, which is prevalent in the Mediterranean region (Cagirgan et al., 2014), it is also a recent phytoplasma-disease symptom that appeared in Fayoum Governorate. According to Duke (1983), the *C. olitorius* plant is particularly problematic in Australia, Egypt, the Philippines, Senegal, Mozambique, and Thailand. Listing the many phytoplasmas pathogens of disease is that notably manifested with signs of deformities as critical issues threatening Egypt's crucial economic crops. Cagirgan et al. (2014) recently reported on the symptomatology of phytoplasma and the incidence of sesame in the same field about climate change or variability. A reported phytoplasma infection in sesame demonstrates the importance of the adjacent wild host of the phytoplasmas infecting plants. Carrigan et al. (2014) stressed the role of weeds in the epidemiology of the disease, and therefore they speculated that the phytoplasma would infect both plants. Ozdemir & Cagirgan (2015) recently documented the phyllody-symptomatology in *C. olitorius* plants with phytoplasma infection in southwestern Turkey.

The nested PCR assays of all the collected samples were tested using universal primer pairs (P1/P7 and R16F2n/R2), and generated fragments of 1271 bp typical to phytoplasma from five symptomatic *C. olitorius* samples. No fragment was obtained from the other samples from non-symptomatic plants also collected from the Fayoum Governorate (Fig. 2). The DNA from a healthy *C. olitorius* plant, used as a negative control, generated no amplicon.

Fig. 1. Leaf deformities, slight yellowing, crinkled leaves, and phyllody symptoms on C. olitorius leaves affected by phytoplasma disease from Fayoum Governorate (A-C) as compared to healthy ones (D)

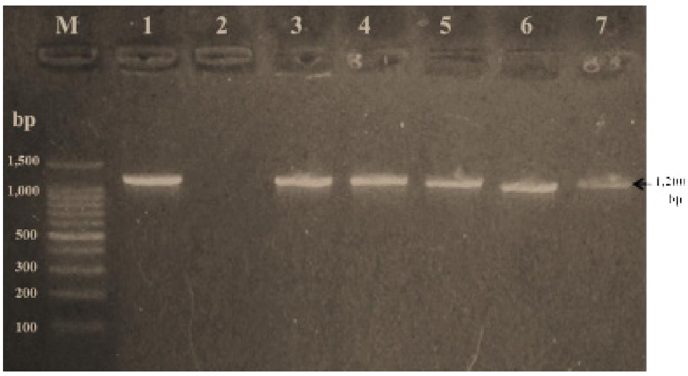

Fig. 2. Gel electrophoresis analysis of nested PCR products amplified from naturally infected C. olitorius infected plants with phytoplasma [M: 100bp marker ladder, L1: positive control, L2: healthy control, L3 to L7 symptomatic samples]

Detection of phytoplasma in *C. olitorius* plants with nested-PCR using R16F2n/R16R2 primers was positive from leaves. Moreover, the amplified product of nested PCR was sequenced and submitted to the GenBank database under the accession number (OQ836595). Phylogenetic analysis was performed based on 16S rDNA in comparison with sequences of the 21 phytoplasma strains listed in GenBank with the isolation from Egypt. Results of phylogenetic analyses indicated that the grouping of our isolate in the phylogenetic clade that encloses the phytoplasma strains belong to group 16SrII (Fig. 3). Based on sequence comparisons, the Egyptian phytoplasma starin was found to share 98.1% sequence identity with *C. olitorius* (ACC. No OP889144 and KM103730) from China and Egypt, respectively, as well as group 16SrII members *Sesamum indicum* (ACC. No OQ437342), *Solanum lycopersicun* (ACC. No MN462969), *Capsicum annuum* (ACC. No MG748735), and *Chrysanthemum morifolium* (ACC. No MF583741) from India. Additionally, based on sequence data, it was found that the Egyptian phytoplasma isolate shared 98% of its nucleotide identity with *C. aestuans* (KX645865) and *cucurbita pepo* (FR822709) from China and Egypt, respectively, and 97.9% of its identity with *C. aestuans* (JX871467), a Chinese species that is classified in the 16SrII group. Our results are in agreement with those of Ozdemir & Cagirgan (2015), Gopala (2018), and Sumashri et al. (2021). So, the identification of the tested strain and its description as a new Candidatus Phytoplasma was carried out.

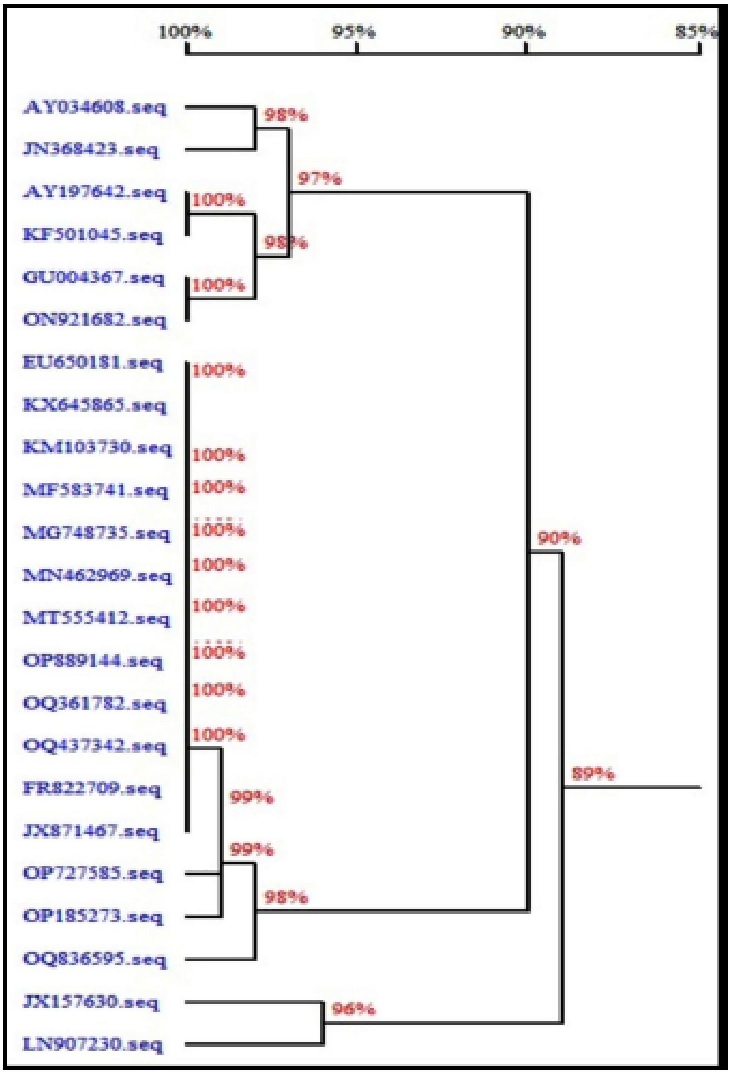

Fig. 3. Phylogenetic tree constructed based on the sequence of the 16S rRNA, showing the phylogenetic relationship of OQ836595 (GenBank accession no. of phytoplasmas isolate in the present study indicated by black triangle) with other phytoplasma sequences belonging to different groups available in the GenBank using DNAMAN ver.7

TABLE 1. Comparison of the nucleotide sequences of the 16S rRNA gene of phytoplasma in the present study (Acc. No. OQ836595) with the corresponding sequences of phytoplasma isolates from Egypt and other countries

Acc. Nos.	Host plants	Country	Group	Identity %
OQ836595	*Corchorus olitorius*	Egypt	16SrII	100
OP889144	*Corchorus olitorius*	Egypt	16SrII	98.1
KM103730	*Corchorus olitorius*	China	16SrII	98.1
OQ437342	*Sesamum indicum*	India	16SrII	98.1
MN462969	*Solanum lycopersicun*	India	16SrII	98.1
MG748735	*Capsicum annuum*	India	16SrII	98.1
MF583741	*Chrysanthemum morifolium*	India	16SrII	98.1
OQ361782	*Glycine max* (soybean)	India	16SrII	98.1
MT555412	*Croton bonplandianus*	India	16SrII	98.1
KX645865	*Corchorus aestuans*	China	16SrII	98.0
EU650181	*Corchorus aestuans*	China	16SrII	98.0
FR822709	Squash	Egypt	16SrII	98.0
JX871467	*Corchorus aestuans*	China	16SrII	97.9
OP727585	*Corchorus olitorius*	Egypt	16SrII	97.4
OP185273	*Sesamum indicum*	Egypt	16SrII	97.4
AY034608	Erigeron	Brazil	16SrVII	89.0
GU004367	Potato	USA	16SrVI	88.6
JN368423	strawberry	Argentina	16SrVII	88.5
AY197642	elm trees	USA	16SrV	88.3
KF501045	*Corchorus olitorius*	India	16SrV	88.3
LN907230	*Hyalestes obsoletus*	Egypt	16SrXII	87.8
JX157630	sugarcane	Egypt	16SrXII	87.7

Chemical analysis test

The results of the chemical examination showed that the tocopherol and crude fiber contents for healthy mallow leaves were 119.96µg/g and 11.32g/100g, respectively, while these values were 117.21µg/g and 15.40g/100g for mallow leaves that were infected by phytoplasma. In this study, infection with phytoplasma changed the amount of some biochemical constituents in the leaves of *C. olitorius* plants, like tocopherols and crude fiber. Antioxidants called tocopherols, which are lipid-soluble, help plants tolerant to abiotic stressors (Kumar et al., 2013). Tocopherol is the primary vitamin E substance found in leaf chloroplasts, where it is present in the thylakoid and chloroplast envelopes. The author hypothesized that a deficiency in alpha tocopherols would affect interactions with phytoplasma, given the importance of lipid peroxidation in plant defense. The tocopherols scavenge lipid peroxyl radicals in thylakoid membranes to deactivate reactive oxygen species produced during photosynthesis and stop the spread of lipid peroxidation (Munné-Bosch, 2005). It is generally assumed that increases in tocopherol contribute to plant stress tolerance, while decreased levels favor oxidative damage (Munné-Bosch, 2005). According to a previous study (Cela et al., 2018), tocopherols may operate in cell signaling to influence processes outside of chloroplasts in addition to their antioxidant action. Additionally, some research indicates that cooking may cause significant vitamin and antioxidant losses (Lin & Chang, 2005; Adebayo, 2010; Saikia & Mahanta, 2013).

Fiber at normal levels is deemed to be a key component in healthy eating and has been shown to have positive benefits on blood cholesterol, the avoidance of gastrointestinal illnesses, and increased glucose tolerance (Adebayo, 2010). The other negative side effect of phytoplasma is the increased level of crude fiber in leaves. The crude fiber content for the leaves of infected mallow plants with phytoplasma was high (0.46%), 15.40g/100g as compared to healthy leaves (11.32g/100g). This outcome is in line with the findings of Kaczmarczyk et al. (2012), who suggested that the capacity of extra fibre to promote the growth of certain phytoplasma was responsible for its effects.

Conclusion

Phyllody disease of mallow (*Corchorus olitorius*) plants was detected in the Fayoum governorate. A related strain of the phytoplasma of *C. olitorius* was recognized as phytoplasmas; it was isolated from Egypt (OQ836595). Phytochemical analysis reveals that healthy leaves possess different phytochemicals and may be used for therapeutic purposes. The effect of Phytoplasma on many active compounds has been identified from *C. olitorius* leaves in the current study. *C. olitorius* plants require special attention to avoid the transfer of phytoplasma to other cultivated economic crops.

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التشخيص والتوصيف الجزيئي للفيتوبلازما وتأثيرها على الفيتامينات والألياف في محصول *Corchorus olitorius* في مصر

عمرو فراج، ايمان الأباجي و سماح مقبل

قسم ابحاث الفيروسات و الفيتوبلازما - معهد بحوث أمراض النبات - مركز البحوث الزراعيه - الجيزه مصر.

تم الكشف عن الفيتوبلازما فى محصول الملوخية *Corchorus olitorius* عام 2022 بمحافظة الفيوم - مصر. ظهرت على نباتات الملوخية المصابة أعراض توريق الأزهار، تشوهات الأوراق وإصفرار طفيف للأوراق. تم إستخلاص الحامض النووى من كلًا من النباتات التى ظهر عليها أعراض والخالية من الأعراض وأختبرت للتأكد من وجود الفيتوبلازما باستخدام أزواج من البوادئ العامة P1/P7&R16F2n/R16R2 كان ناتج التضاعف لهذه البادئات حوالى 1271 زوج من القواعد، فى حين لم ينتج أى تضاعف للحامض النووى DNA المستخلص من النباتات التى لم يظهر عليها أعراض. تم عمل تتابع نيوكلتيدى لناتج nested PCR باستخدام البادئات R16F2n/R16R2 وخضع هذا التتابع لدراسة درجة القرابة باستخدام برنامج DNA-man. أشارت نتائج الدراسة اعتمادًا على عزلة الفيتوبلازما محل الدراسة والمسجلة على البنك القومي للجينات برقم (Acc. No OQ836595) درجة قرابه 98.1% مع عزلات الفيتوبلازما (OP889144 and KM103730) من مصر والصين ومحاصيل أخرى بالهند ولاتزال هناك حاجة لتعريف نوع الناقل الحشري المسئول عن إنتشار الفيتوبلازما في نبات الملوخية. هناك العديد من المركبات الكيميائية الفعالة الموجودة في نبات الملوخية لها أهمية كبيرة نظرًا لقدرتها على تحسين صحة الإنسان. قد اكدت هذه الدراسة أن وجود الفيتوبلازما في أوراق النباتات المصابة أدى لإنخفاض محتواها من الـتوكوفيرول (Vitamin E) وزيادة محتواها من الألياف.