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The involvement of the vital secondary metabolites of the pathogen *Alternaria alternate* (Fr.) Keissl. in the occurrence and progression of Alternaria leaf spot disease in date palm (*Phoenix dactylifera* L.)

Review Article

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Abstract

Leaf spot disease is one of the most significant and pervasive date palm diseases in the Basrah region of Iraq. The symptoms appear on the leaves of the fronds and on the medial veins of the fronds of the date palm trees. Different colors and shapes of spotting symptoms depend on the causative fungus and the environmental conditions. There are several potential causes of leaf spot disease, but one of the most significant is the fungus *Alternaria alternata* (Fr.) Keissl. *A. alternata* is essentially a saprophytic, global fungus that enters epidermal cells directly due to its ability to be pathogenic. It is crucial to research how their pathogenicity has developed within the host plant. *A. alternata* isolates directly attack the host plants by piercing the fungal hyphae using small infection pegs that enter the susceptible host plant, such as the fronds (rachis) of date palm trees, and the infection symptoms have shown as erratic brown dots. These spots enlarge throughout this time, turning the tissue brown and possibly turning the core of the spot pale white, and in cases of severe infection, the death of leaves occurs. *Alternaria alternate* is a highly metabolically active fungus, and as a result, the metabolic by-products of this fungus are strongly related to plant disease. Metabolic products play a crucial role in the ability of fungi to withstand a variety of adverse environmental circumstances. From these starting points, the following review seeks to shed some light on certain specifics about the biology, pathogenicity, physical traits, and significant secreted metabolites of the hosts of this fungus.

Keywords: Alternaria alternate, date palm, leaf spot, metabolic products, pathogenicity

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Introduction

For many years, date palms (*Phoenix dactylifera* L.) have been grown in the oasis in the Arab desert. Additionally, date palm trees have a long history of cultivation and use in the Middle East and North Africa dating back more than 5,000 years, and they play a significant role in sustainable agriculture in many nations throughout the world (Al-Khayri, 2007). Their fruit is rich in vitamins, minerals, fiber, and sources of carbs. Their fruits can also be used medicinally since they contain several chemical substances, such as procyanidins, sterols, carotenoids, phenolics, and anthocyanins, that have a positive impact on health. The fungal pathogen is one of the major causes that affects the setting of dates and palms (Abdullah et al. 2010). Ammar and El-Naggar (2011) stated that one of the most significant and pervasive date palm diseases in the Najran region of Saudi Arabia is the leaf spot disease, which is quite common. According to other investigations by Livingston et al. (2005), one of the causes of threats to palm cultivation in recent years has been leaf spot infections. Numerous studies have shown that there are several potential causes of spot disease, but one of the most significant is the fungus *Phoma* spp. (El Hadrami and Al-Khayri, 2012; Abd Al-Hseen and Manea, 2020). Zaid et al. (2002) showed that this fungus causes date palm leaf spot disease in the recent past, although there are no references in Iraq that describe it as a pathogen on plants, and the most recent thorough review and new information about date palm infections have surfaced. This review aims to provide updated information about the biology, epidemiology, and pathogenicity of the fungus *Alternaria alternata* the causative pathogen of Alternaria leaf spot disease that has been reported on date palm (*Phoenix dactylifera* L.).

Symptoms, Pathogenicity, and Epidemiology

On the fronds, the symptoms have shown as erratic brown dots (rachis). During this time, these blotches grew larger and turned the color brown. This spot's center was turning a light shade of white in the meantime. On the other hand, the spot surface could be depressed below the tissue's surface. In general, lower whorls and older leaves have more severe infections than upper, younger leaves, and the severity and frequency of infections increase with increasing palm age. On green leaves, the symptoms appear on the middle veins of the fronds as oblong-shaped spots with pale brown colors tending to gray, and their edges are characterized by dark brown and reddish centers from which the fungus is always isolated. Date palm trees in Iraq and all countries that grow date palms are frequently affected by leaf spot diseases, and there are numerous fungal

species have been discovered on palm leaves that exhibit the signs of leaf spot. (Carpenter and Elmer, 1978; Livingston et al. 2002). The most prevalent of these diseases is brown leaf spot, which is the brought on by *Alternaria alternate*. The fungus pathogen was isolated and diagnosed from date palm trees that showed symptoms of spot disease on the fronds (Al-Zubaidi, 2005).The pathogenicity assay for the fungus *A. alternata* showed spotting symptoms when artificial infection was carried out on the middle race of the fronds (Jassim, 2017). Other fungi that cause leaf spot symptoms on palm trees include *Bipolaris australiensis*, *Drechslera* sp., *Helmnthosporium* sp., *Colletotrichum* sp., *Stemphylium* sp., *Pestalotiopsis palmarum* , *Chaetosphaeria* sp., *Phomopsis* sp., *Phoma* spp., (Livengston et al. 2002; AL-Zubaydi, 2005; El Deeb et al. 2007; Carpenter and Elmer, 1978). Spotting symptoms generally focused on the last leaves in the tree, meaning that the mature leaves and old ones are the most sensitive to the infection with leaf spot pathogens because those leaves are prepared to host most of the fungi whose names were written above. It was also found that the severity of the infection often increases with the age of the tree. It is worth noting that the tree is usually susceptible to attack by pathogens that cause spotting symptoms, therefore we may find different forms of spots on one leaf. For the purpose of identifying the causal fungus of leaf spots, it is necessary to diagnose the causal agent because the availability of appropriate environmental conditions for all pathogens may cause the areas of infection to interfere and the symptoms of spots to be similar. Samples of infected leaflets with a limited number of spots can be collected and then pieces of the leaf are superficially sterilized and kept moistened glass dishes to allow the spot to produce structures of fungal hyphae and conidia carriers as well as spores for the purpose of diagnosis. The microscopic investigations found that mycelium was septate and branched, conidia are formed singly at the tip of the conidiophores, but occasionally, they are produced in the short chain. On PDA media, *Alternaria alternata* growth colonies developed light green to greenish-dark color mycelia, and at the beginning of the development phase, the colonies were surrounded by white immature tip hyphae that eventually turned greenish-black in color. In the date palm groves closest to the river banks, such as those in Shaat-Al-Arab, where there is a high level of humidity, the majority of fungal diseases have been found in Basra province. Furthermore, date palm leaf spot disease was caused by fungi, which are well-known as genuine pathogens (Carpenter and Elmer, 1978). The genus Alternaria is one of the highly prevalent fungi in nature, and it is belongs to the imperfect fungi, the phylum Hyphomycetes (Ainsworth et al.1973). The genus Alternaria emergence of polymorphic conidia and it is unique. The soil and environment are

both filled with the spores of these polyphagous fungi, and the elongated structure of the conidia makes it simple for the wind to disperse them, increasing their dispersal (Mamgain et al., 2013).The fungus was found in nature in two forms, saprophyte, and endophyte. endophyte form causes different diseases in plants, and there are about 300 types of Alternaria recorded so far (Lee et al.2015). These include *Alternaria alternata*, *Alternaria arborescens*, *Alternaria radicina*, *Alternaria brassicola*, *Alternaria brassicae*, and *Alternaria infectoria* (Lee et al.2015; Tomma, 2003). *A. alternate* causes disease in a variety of economically important plants, including brochelli, tomato, chili, potato, citrus, apple, and others (Meena et al.2016). Leaf spots and defoliation of nursery plants are caused by a variety of fungal diseases, but *Alternaria* spp. is likely the most common and problematic. Plants rarely die from Alternaria leaf spots infection, although they can lower their resistance to pathogens and commercial worth. (Mamgain et al., 2013). Infections with *Alternaria* spp., can result in lesions on the stem and radicle, as well as leaf spots or blotches, early shoot blighting, and blackening. The spots or blotches that appear on affected leaves are brown to black and can vary greatly in size, color, and form. These leaves could drop off too soon. One such species is *Alternaria alternate* (Fries) Keissl., which can be discovered all around the world in the soil and in the tissues of numerous plants. Numerous plant species in various habitats may be harmed with infection by this fungus pathogen(Bagherabadi et al., 2015). *A. alternata* pathovars in particular rely on the generation of toxins to settle on their host. Some of these release toxins that travel via the vascular system causing chlorosis along the veins from the leaf lesion and killing nearby leaf tissue, and there creates a toxin that has a modest phytotoxic impact, as seen in the case of a yellow halo encircling a necrotic region (Pegg et al., 2014). Typically, when leaves are infected, chlorosis and necrotic regions form, which reduces the amount of photosynthetic assimilates produced (Ciuffetti et al., 2010). This decrease in photosynthesis can be related to a pathogen's destructive behavior (Chen et al., 2005). Numerous types of shrubs are affected by some diseases, and their occurrence is rising. Due to their frequency and genetic diversity, Alternaria fungus pose a serious hazard to plant production (Kakvan et al., 2012). According to Goetz and Dugan (2006), *Alternaria* spp. are harmful to coniferous plants and can be found in their rhizospheres. Pomerleau and Nadeau (1960) identified *Alternaria* spp. fungi as the culprit for the withering of 20–65% of fir seedlings in nurseries. Similar results were documented by James and Woo (1987), discovered that seedlings of coniferous plants treated with *A. alternate* totally wilted away. Rodino et al. (2014) found that *A. alternata* on a variety of horticultural and agricultural crops, as well as on several weeds and

ornamentals. According to Sharma et al. (2013), investigations of the genomic DNA from isolates of *A. alternata* from diverse host plants indicated a high degree of genetic differentiation that was advantageous for the colonization of various plant species. The major question that needs to be answered soon is if these poisons produced by *A. alternate* can have to be employed as probes for quick screening of plant clones or the offspring from crossings for the production of disease-resistant plant types. These toxins might function as antibiotics and have to be used to biologically regulate harmful microorganisms, and these toxins might create disease-free plants for future generations soon (Meena and Samal, 2019). Many authors such as Nasim et al.(2012), and Pegg et al.(2014) have revealed that *Alternaria* spp., can infect the shoots and roots system of ornamental plants. *Alternata alternata* may rapidly adapt to closely similar host plants and can be found all over the world in a variety of environmental circumstances (Grunden et al., 2001; Pusz,2009). In 1933, the first Due to the *A. alternate* high metabolic activity, its metabolic by products have a close relationship with phytopathogenicity. As a result, this phytopathogen damages crops, vegetables, and fruits at a cost. *Alternaria* spp., pathogens have been identified to produce more than 70 poisons. and there are two crucial traits of *A. alternate*, one of these is the manufacture of melanin and several host-specific and non-host-specific toxins(HSTs and Non-HSTs). Toxins which were host specific with low molecular weight are common in seven *Alternaria* spp., and these secondary metabolites influence the pathogenicity of the pathogen on the hosts (Barkai Golan, 2008). There are several species of the genus Alternata, which can be found growing in various conditions and come from various regions of the world (Simmons, 1992). The derivatives of cellulose enzymes and other analytical enzymes found in this species of saprophytic fungi play a significant part in the natural processes of organic matter breakdown. However, opportunistic pathogens make up a sizeable fraction of them. Therefore, economically significant plants, such as legumes, vegetables, and fruits, are plagued by a variety of diseases. Because of this, it is extremely challenging to control or eradicate this fungus(Agrios, 2005; Thomma, 2003). The most significant post-harvest pathogens are those belonging to the Alternaria genus. They can infect fruits and vegetables, and many of them are capable of creating toxic fungi that can harm both people and animals while they are infected (Apangu et al., 2018; Stocco et al., 2019).

Secondary Metabolism: The phrase "secondary metabolism" refers to a broad range of metabolic processes that result in the creation of metabolites that appear to be necessary for an

organism's survival, but that may be eliminated without having any discernible impact on that organism's life (Fox and Howlett, 2008). These mechanisms are efficient at reducing or halting the growth of the organism. The middle components of the main metabolic pathway serve as precursors for other pathways, which in turn create crucial molecules of life, such as proteins, nucleic acids, and amino acids. Another primary metabolism does not employ other simple chemicals (intermediates), but rather converts them to other metabolic products. Because of this, secondary metabolism is also known as the "font conversion pathway" (shunt metabolism) (Deacon, 1980). Secondary metabolism is a structural process that consumes energy and results in the synthesis of specialized compounds. These compounds are not required for the growth and development of the organism, and certain species and strains of the organism have developed specializations in this process. The primary metabolite citric acid, which is produced in excess in fungi, is one of these compounds. It is derived from primary metabolic intermediates via distinct pathways (Dube, 1983). In fungi, there are three stages of growth: the equilibrium phase (balanced phase), during which the fungus growth accelerates and nutrient amounts are moderately consumed; fat and carbohydrates are gathered when nutrients run out and the storage phase begins. According to the data, the production of dry mass, constant mass, and metabolites grew until the medium was depleted. These indicators indicate that an organism has entered the maintenance stage, also known as the phase that considers the creation of secondary metabolites. Many studies focused on the balance and storage phase (trophophase), during which the fungus grows without the formation of secondary metabolites. This phase ends when the medium runs out of basic nutrients like nitrogen and phosphorus, at which point the fungus enters the inactive or stagnant (idiophase) phase, where secondary metabolites are produced as well as the beginnings of disintegration (Dube, 1983). Consequently, in accordance with the idiophase, some researchers referred to secondary metabolites as "idiolites" (Demain, 1986). To avoid being harmed by the primary metabolites' intermediary chemical substances that had accumulated in its cells, nutrient termination runs a variety of metabolic pathways. This conversion from primary metabolism to secondary metabolism also prevents sporogenesis or conidiogenesis in fungi. The majority of secondary metabolites are commercially valuable substances like antibiotics, but some of them are toxic to both humans and animals, such as mycotoxins (Isaac, 1997). The secondary metabolites are grouped into groups based on the source of the biosynthesis, despite the vast variability in their chemical structure. Low molecular-weight compounds, such as nucleotides, amino acids, sugars, acetyl-CoA, and related substances involving TCA cycle

intermediates like tricarboxylic acids and terpenes, are categorized into a small number of groups, which is reflective of their role as the raw materials for cell components. The protein biosynthesis routes used in the creation of cellular components like nucleotides, hemi, and sterols are pumped with a number of amino acids by microorganisms as they expand. Since genetic studies are crucial to the investigation of secondary metabolic pathways, researchers are attempting to arrive at genetic information either selectively or in other ways to promote the development of secondary metabolic materials of interest in medicine (Craney et al., 2013). The metabolic grid network is created when two separate enzymes can convert the same intermediate, which is primarily the case with secondary metabolism enzymes (Dube, 1983). The secondary metabolism-related genes in fungi form clusters and often consist of two or more genes that walk through the metabolic pathway while encoding enzymes, transcription factors, and vectors (Keller and Hohn, 1997; Woo et al., 2010). The cells of the fungal mycelium produce specific toxins like AOH and AME, which are produced in the newly developing conidia, and it is unknown whether such a distinctive feature is found in *Altrnaria* spp., or other fungi. The secondary metabolic pathway of *A. alternata* also works in conidia and does not only track on somatic hyphae cells. There are seven patterns (pathotypes) in *A. alternata*, and each one produces a toxin specific to the HSTs of its host. (Häggblom, 1987; Ito et al., 2004). Secondary metabolism plays a vital role not only in pathogenicity, such as the production of mycotoxins, environmental protection, and competition with other species, but also in the various stages of fungal development. After fungus development is complete, the bulk of secondary metabolites are created, and this is when the crucial sporogenesis stage begins (Calvo et al., 2002). Andersen and Thrane in 1996 isolated a group of *A. alternata* from *A. infectoria* through the production of various metabolites from each. *A. alternata* produces many metabolites such as alternariol, AME, AOH, altertoxin with two groups I and II, and TeA. The *A. infectoria* group produces a variety of unknown metabolites that were detected by HPIC-DAD (Andersen and Thrane 1996).

Toxins produce by *Alternaria alternate*:

Mycotoxins are organic substances that are produced by secondary metabolism and are not directly required for fungal development. Nearly 80% of the secondary metabolites produced by *A. alternata* are mycotoxins, which allow host defenses to enter and kill target cells while obtaining the nutrients required for growth and other essential functions (Howlett, 2006). *A. alternata* has multiple pathotypes that target various plants, and each one makes unique

mycotoxins for each host. Examples of these toxins and their hosts include the AK toxin, which causes a black spot on Japanese pear fruit; the AAL toxin, produced by a pathotype that causes tomato trunk warts; the AF toxin, which affects strawberries; the AM toxin, which develops from a pathotype that affects apples; the ACT toxin, produced by the tangerine pathogen; and the ACL toxin, produced by the rough lemon pathogen (Agrios, 2005). The molecular study of the toxins produced by *A. alternata*, which range from secondary metabolites with low molecular weight to peptides, supported the notion of the pathotypes of this organism (Kusaba and Tsuge, 1997). At varying concentrations, *A. alternata* produces all or a portion of the toxins grouped above, but at one of them is higher than the others. As a result, these poisons were sequenced by varying host concentrations (Guo et al., 2019, Noser et al., 2011, Patriarca et al., 2007, Scott et al., 2012). Additionally, a single isolate of *A. alternata* has the ability to infect two distinct hosts by generating two distinct HSTs (Masunaka et al., 2005). ACT toxins, which are found in a cluster on a single tiny chromosome known as a dispensable chromosome, are just one of the toxins which have genes. This chromosome is not necessary for development, but becomes so under certain conditions that it is important for growth, and it is the cause of infection and rot in young plant leaves and fruits (Stuart et al., 2009). Toxins that act toward multiple objectives or just one within a single cell will ultimately cause plants to perish. The plasma membrane is infected by the ACT, AF, and ACTG toxins, which results in a loss of permeability. Although the destruction caused by the AM toxin's thylakoid inhibitory electron chain and oxygen transfer rates on the chloroplast and plasma membrane also has an effect on the inhibition of photosynthesis (Dai et al., 2004; Kohmoto et al., 1984; Otani et al., 1995), The mitochondria are affected by ACT and AT toxins, whereas ACR causes the mitochondria to bulge, alters its morphology, and enhances NADH oxidation. This causes the plasma membrane regularity to decline, which causes electrolyte loss and necrosis (Akimitsu et al., 1989; Otani et al., 1995). Toxins produced by fungi may or may not be specific to a particular host, depending on their level of specificity. Toxins that are unique to anything not only support the pathogenicity of the cause, just like virulence factors and enzymatic activities do (Ballio, 1991). The most effective method to classify mycotoxins in general, or the toxins produced by the fungus *Alternaria* spp., in particular are chromatographic techniques like UPLC (Noser et al., 2011). Some of these toxins have also been found in hepatocytes at doses of 50 mM using PCR (Hessel-Pras et al., 2019). Numerous non-specific poisons produced by various species of Altrnaria, such as tenuazonic acid, TEN, and zinniol, have been the subject of in-depth research. Many hypotheses have been put forth to

explain the harmful effects of these poisons, among other things. Tenuazonic acid slows protein synthesis, and curvularin prevents cell division by inhibiting glycolysis of microtubules; zinniol affects membrane permeability (Robenson and Strobel, 1981; Thuleau et al., 1988).Genetic mutations are crucial for understanding the function of toxins in pathogens or virulence because they enable the dissection of biosynthetic pathways by gathering data on the gene sequences that code and produce these toxins (Howlett, 2006; Keller et al., 2005).

The Melanin

Melanin is a dark pigment that is black in color and is produced by all living things, including higher species like humans. Although melanin is not required for development or evolution, it does help the species remain competitive and viable and protects organisms from the challenges of the environment. Additionally, it can make the pathogenic organisms that are carrying it more virulent (Jacobson, 2000). The melanin in fungi is found in the cellular walls of both hyphae and spores and is referred to as "melanin attached to the wall" in these instances ("wall-bound melanin"). The fungus melanin may be found in fibers or phenolic granules extruded outside of the cell wall, where it will later be subjected to external oxidation by the fungus enzymes (Bell and Wheeler, 1986; Nosanchuk et al., 2015). According to research using electron microscopy and other methods, *A. alternata* produces melanin, which it then accumulates in the cell walls of the fungal mycelium, namely in the cell walls of conidia (Carzaniga et al., 2002; Kheder et al., 2012). Most plant pathogenic fungi have appressoria, which are structures that help them penetrate plant cell walls. As with *M. grisea* and other pathogenic fungi, it is thought that melanin aids these appressoria performing their functions, and many people view it as a virulence factor (Jacobson, 2000; Kheder et al., 2012). Because the appressoria of *A. alternata* do not contain melanin, it has been assumed that melanin has no relation to pathogenicity. Because of these facts, numerous studies have focused on melanin as the primary cause of fungal pathogenicity using a variety of techniques, including biological controls like the use of bacteria or fungi that can stop the production of melanin or the use of worms and other small animals that eat those melanized fungi (Butler et al., 2005). Melanin serves as an antioxidant because of the numerous methyl groups that are present in its structure and stop the peroxidase enzyme from producing oxidative intermediates (Shcherba et al., 2000). The melanized wild type of *A. alternata* was able to equalize oxidizing permanganate, hypochlorite, or hydrogen peroxide, while the albino mutants were unable to do so (Jacobson et al., 1995).

Disease control

Fungicides Mancozeb + copper can be sprayed on date palm leaves to effectively manage the disease in early stages. Old infected leaves of date palm should also be removed and burned right away once a year (Zaid et al.2002; Livingston et al.2002).

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المنتجات الايضية الثانوية التي ينتجها الفطر *Alternaria alternate*(Fr.)Kessiel وأهميتها في حدوث وتطور الاصابة بمرض التبقع الالترناري في نخيل التمر(*Phoenix dictylefera* L.)

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الخلاصة

يعد مرض تبقع الاوراق على اشجار نخيل التمر من اكثر الامراض أهمية و انتشارا في بساتين نخيل التمر في محافظة البصرة /العراق. تظهر الاعراض المرضية على الوريقات والعرق الوسطي(الجريد) لسعف النخيل على هيئة بقع مختلفة الاشكال والالوان وهذا يعتمد على الفطر المسبب والظروف البيئية. هناك العديد من العوامل التي تؤثر في حدوث أمراض التبقع حيث يعتبر الفطر الممرض من أهمها. يعتبر الفطر *Alternaria alternate*(Fr.)Keissl. أحد الفطريات المترممة الواسعة الانتشار في الطبيعة, يستطيع الفطر أحداث الاصابة عن طريق الاختراق المباشر لخلايا البشرة وذلك لقدرته العالية في احداث المرض وهذا يجعل من الاهمية بمكان البحث عن الكيفية التي يستطيع الفطر الممرض مهاجمة النبات العائل. أن عزلات الفطر *A. alternata* تستطيع مهاجمة عوائل النباتات الحساسة بواسطة الخيوط الفطرية التي تكون في نهاياتها تراكيب صغيرة تسمى وسائد الاصابة حيث تستطيع هذه التراكيب اختراق خلايا النبات العائل واحداث الاصابة الاولية التي تكون على شكل بقع صغيرة بنية غير منتظمة، تكبر هذه البقع بمرور الوقت لتشمل معظم أو كل أجزاء الورقة ويتحول النسيج المصاب الى اللون البني كما تكون مراكز هذه البقع ذات لون ابيض نتيجة لموت الانسجة فيها، و في حالات الاصابة الشديدة تموت الاوراق. للفطر *A. alternata* نشاط أيضي قوي ونتيجة لذلك فأن المنتجات الايضية لهذا الفطر ترتبط ارتباط وثيق بأمراض النبات وتلعب دور مهم في قدرة الفطر على أحداث الاصابة في العوائل النباتية المضيفة و على تحمل الظروف البيئية القاسية والمتطرفة. تسعى هذه المراجعة الى القاء الضوء على بعض التفاصيل المحددة حول حياتية و إمراضيه الفطر والصفات الظاهرية والمنتجات الايضية المهمة التي ينتجها هذا الفطر الممرض وأهميتها في حدوث وتطور الاصابات المرضية.

الكلمات المفتاحية: Alternaria alternate ، نخيل التمر، تبقع الاوراق، ايض ثانوي، إمراضيه