Original article

Isolation and Classification of Fungal Pathogens of Salvia Rosemary in Different Regions of Al Jabal Al Akhdar

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Abstract
Rosemary trees are considered one of the most important natural flora in Libya, which represents the total vegetation cover prevailing in the natural forests of the Green Mountain region, which has currently been subjected to the phenomenon of sudden death, which has led to a change in their density and distribution, threatening their survival. The current study aims to isolate and classify the fungi that cause rosemary diseases in the areas of the Green Mountain, Shahat and Slanta, seeds and stems. Samples were collected from the seeds and stems of the plants in April 2023, on which we found the infection. The samples were retention in sterile bags and retention in refrigerated until they were transported to the laboratory to be analyzed. Types of fungi have appeared10, *Aspergillus flavus, Aspergillus niger, Aspergillus fumigates, Aspergillus thermomutants, Alternaria Sp, A.altenate, Apophomyces variabilis Pencillium Sp, Pencillium chrysogenum, Mucer Sp.*
Keywords. Rosemary, Aspergillus flavus, Aspergillus niger, Aspergillus fumigates.

Introduction

Salvia rosmarinus, a native Mediterranean shrub, has a strong aroma. scent, and its leaves are dark green, long and white. Its flowers are blue or purple [1-2]. The name used scientifically is Salvia rosmarinus, S. rosmarinus is considered an aromatic plant, and oil is extracted from it, which has a protective effect against the degradation and reduction of lipid oxidation. It is used as a natural preservative for foodstuffs [5-6]. It is used medically to wash wounds by boiling rosemary with vinegar, due to its soothing and antiseptic properties [7]. It is one of the trees that can withstand winter temperatures of approximately -20 degrees Celsius. It can overcome severe water shortages in the long term (7).

Rosemary contains antifungal, antiviral, antibacterial, anti-inflammatory, and antioxidant properties [8-10]. It is considered an invasive species. This plant is characterized by a slow growth rate and a lifespan of up to 35 years [1]. The leaves are evergreen, 2–4 cm (3⁄4–1+1⁄2 in) long and 2–5 mm (1⁄16–3⁄16 in) wide, green above, white below, with dense, short, woolly hairs [1-2]. The plant blooms in spring and summer in temperate climates, but the plant can be in continuous bloom in warm climates. The flowers are white, pink, purple, or dark blue. The branches are arranged in clusters of 2 to 3 flowers along their length.[7] Rosemary also tends to bloom outside of its normal flowering season; It is known to flower in late December and as early as mid-February (in the Northern Hemisphere) [7]. The study aimed to isolate and classify the fungi that cause rosemary plant disease in different areas of the Green Mountain (Shahat and Salantah). The study was on the seeds and stems of the plant.

Methods

Study area

Shahat is a historical city founded by the Greeks on the Green Mountain in the far north-east of Libya. It is about 10 km away from the city of Al-Bayda. Shahat is considered the second largest city in the Green Mountain Governorate after the city of Al-Bayda. Slanta is a town located in the Green Mountains, about 27 km south of the city of Al Bayda. The study included the slanta forests and the Shahat forests, as one of the most important areas of the Al Jabal Al Akhdar in which rosemary trees grow, which showed fungal infections and death of the growing tops of the rosemary plant.

Samples collection

Samples were collected from the seeds and stems of the plants in April 2023, on which we found the infection. The samples were retention in sterile bags and retention in the refrigerator until they were transported to the laboratory to be analyzed**.**

Isolation and classification of pathogenic fungi

The plant was cleaned with water to remove dust and dirt, and was cut into parts less than 10 mm. We had a healthy part and an infected part from the same sample, and then soaked them in sodium hydrochloric acid at a concentration of 5%. After that, the plants were washed with sterile distilled water several times to remove the remains of sodium hydrochloric, and then we dried the plants by placing them on sterile filter papers. After the drying process, using sterile tweezers, we place the samples on environmental dishes with the addition of 0.0005 g/L of chloramphenicol, and after the environment used is cooled, the pH is adjusted to 5.2 using a device made in pH meter by adding a 1N hydrochloric solution [laboni 1990] As for seed samples, the seeds are placed after grinding the fruits between two filter papers and placed on an

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environmental medium. We place all samples at a temperature of 28°C +_ 1, where each sample has three replicates. Place it in incubation for 2-7 days until fungal growths appear clearly on the plate. Pure cultures are obtained for all samples using hypha tip isolation or single bacterium isolation, Samples are preserved until identification. Fungal isolates are identified according to [11-12-13] based on the morphological characteristics of fungal culture.

Fungal isolates were identified according to [11-12-13] based on the morphological characteristics of the fungal culture, using direct examination with a microscope and using octophenol blue dye. The infected seeds were planted after being soaked in hot water for 10 minutes in petri dishes containing filter paper and incubated for three weeks.

Results

The results are shown in the following figure (1): The appearance of 10 species from five fungal genera in the stems of rosemary plants in the forests of Shahat and Slanta. This figure shows the fungi that appeared on the seeds of the rosemary plant in the areas of Shahat and Slanta**.**. The highest percentage of the fungus *Mucer sp* was in the forests of Shahat and its percentage was 27% in the forests of Slanta. Then the *Aspergillus fumigates* fungus was at a rate of 17% in the forests of Slanta and at a rate of 14.5% in the forests of Shahat, then the fungus *Aspergillus flavus* was at a rate of 13.5% in the forests of Slanta and at a rate of 3% in the forests of Shahat, then the fungus *Apophomyces variabilis* was in the forests of Shahat at a rate of 12.2% and did not appear in the forests of Slanta. Then *Aspergillus niger* did not appear in the forests of Slanta and appeared at a rate of 6% in the forests of Shahat, and the fungus *Aspergillus thermomutans* appeared at a rate of 5% in the forests of Shahat and did not appear in the forests of Slanta, then the fungus *Pencillium sp* appeared at a rate of 6% in the forests of Shahat and did not appear in the forests of Slanta, then the fungus *A.altenate* appeared at a rate of 7.8% in the forests of Slanta and 4% in the forests of Shahat.

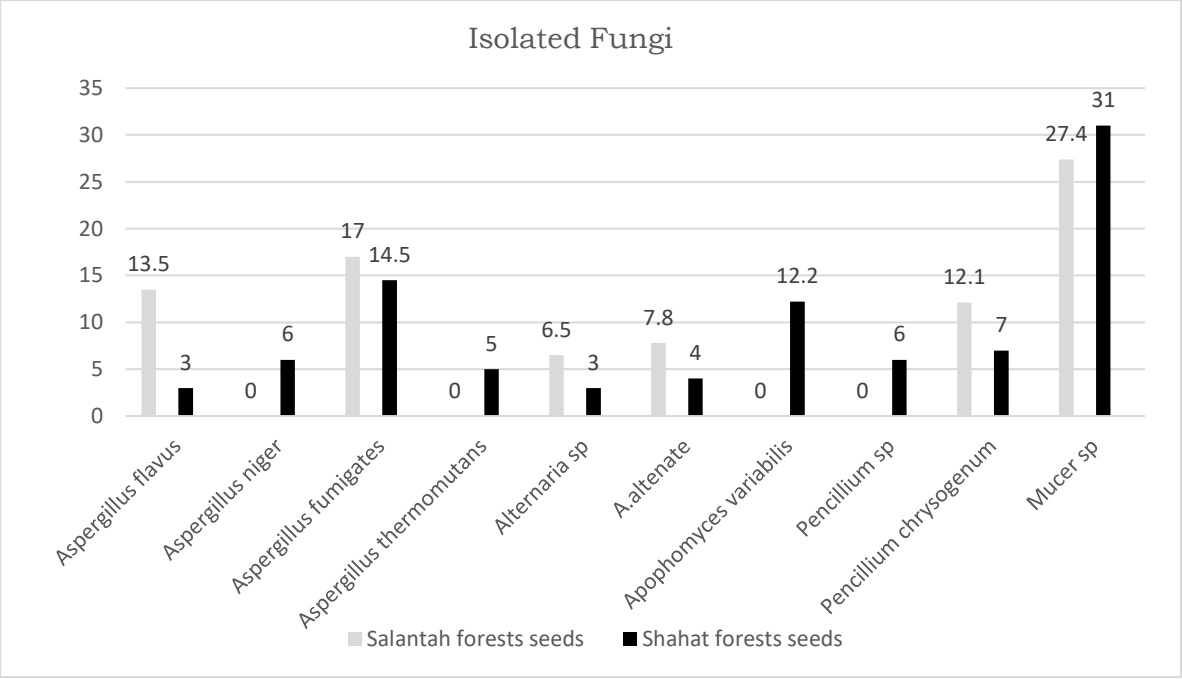

Figure 1. The spread of pathogenic fungi on seeds of rosemary Salvia plants.

Figure 2 shows some of the fungi that appeared on the stems of the rosemary plant in the areas of Slanta and Shahat. We noticed that some of the fungi that appeared on seeds in the same plant did not appear, and the highest percentage was for the fungus *Mucer sp*. in Shahat, at a rate of 27.4%, and in Slanta, at a rate of 7%. Then, the fungus *Aspergillus fumigates*, at a rate of 9.1%, in the forests of Slanta and did not appear in the forests of Shahat. Then, the fungus *Pencillium sp*. appeared at a rate of 3% in the forests of Shahat, but did not appear in Shahat. Slanta forests, and then the *Aspergillus thermomutans* fungus appeared at a rate of 2% in Shahat forests and did not appear in Slanta forests.

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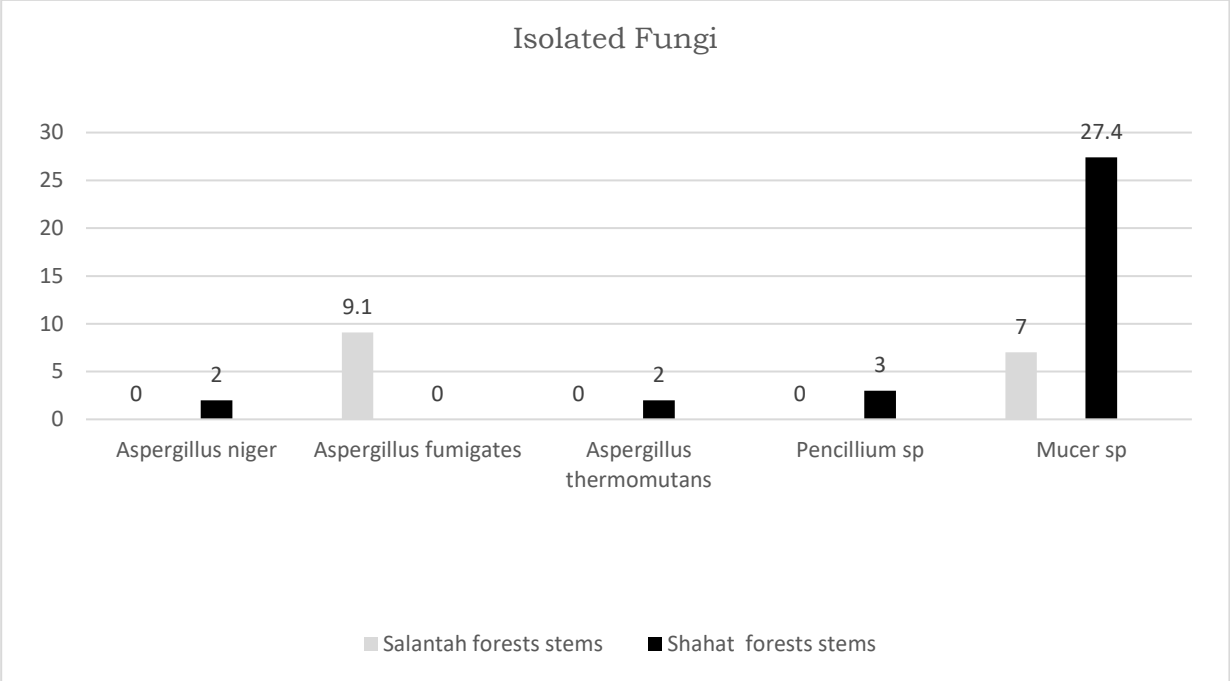

Figure 2. The spread of pathogenic fungi on the stems of the rosemary plant Salvia

Discussion

The study showed that the infection sites in these forests differ among themselves in terms of the appearance of symptoms and the degree of infection. It was noted that the samples taken from rosemary seeds in these areas amounted to 176 fungal isolates, while the fungal isolates taken from the stems amounted to about 57 fungal isolates. These results are consistent with [14] who isolated fungi associated with corona forests in the of Saudi Arabia. Rosemary seeds contain a greater percentage of rosemary stems than rosemary plants.

The results showed that fungal species *Asperigillus sp* Clear growth in all forests. This may be due to the genetic methods of these fungi to avoid the host's defenses [15]. Also, the presence of *Asperigillus, sp*. species in the dust particles in the Khamasin winds that blow over Egypt and Libya in the summer periods during July and August may contribute to the density of the spread of this fungus, and this agreed [16], where it was observed that rosemary trees deteriorated after the dry Khamseen winds, carrying with dirt and dust. It was also shown that the *mucer, sp* fungus was accompanying the seeds and stems in all the forests subject to the study, where the highest percentage was prevalent in the seeds between (27-31) in all the forests, where the reason for the spread of this fungus in the study areas and the higher percentage in the seeds may be due to the presence of *asperigillus* spores of the *mucer. Sp.* The spread of this fungus in the study areas and its high percentage in seeds are due to the presence of *Aspergillus* spores of the myxomycete fungus. sp on infected fruits that fall on the soil surface.

The *Alternaria sp* fungus also appeared on rosemary plants, which is considered one of the fungi that causes stem rot disease near the surface of the soil and causes the appearance of spots and blights on the vegetative and pink shoots of the plants that reach the fruits [17], also added that the *Alternaria sp* fungus effects on newly grown seedlings, it appears in reddish-brown on the branches, then complete death of the seedlings occurs.

Conflict of interest. Nil

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