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An *Alternaria* Leaf Spot of the Swiss Chard in Tripoli- Libya

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Abstract

This research was conducted to study the disease incidence (DI%) and disease severity (DS%) of the Swiss Chard disease caused by *Alternaria alternata* on Swiss Chard crops at four sites of Tripoli; Soq – Aljumma, Al-ghrarat, Al- Harart and An-Nofleen. A total of 100 plant samples were collected during this study. Results showed that percentage of disease incidence and disease severity varies among the surveyed locations. The results also showed that the five selected Beta vulgaris yielded different susceptibility. Barese was the high susceptible to the pathogen in (DI %) and (DS %), under greenhouse condition compared to control, whilst, Orange Fantasia was the lowest susceptible. In addition to that, Fordhook Giant was high susceptible under field conditions, also Celery was less favorable under greenhouse conditions compared to others. Parsley was less favorable under field conditions compared to other hosts tested. *Alternaria alternata* has a wide host range include all tested plants with different (DI%) and (DS%).

Key words: Swiss chard disease, *Beta vulgaris*, disease incidence, disease severity, *Alternaria alternata*.

Introduction:

The genus *Alternaria* is one of the Deuteromycetes fungi was first described by Nees (1816). It includes saprophytic, endophytic and plant pathogenic species differentiated by the formation of polymorphous conidia in single or in short or longer chains and provided with cross, longitudinal as well as oblique septa and having longer or short beaks. The spores of this fungus found commonly in the atmosphere and also in the soil (Neeraj & Verma, 2010). *Alternaria* spp. can cause severe problems in agriculture by constriction crop yields, and so cause big economic losses to farmers (Garg & Singh, 2016). Symptoms of disease were dark brown circular or irregular spots on healthy plants. Initially, the lesions were confined within distinct parallel veins of the leaves, but eventually expanded and coalesced across veins. *Alternaria* causes huge economic losses due to their wide host range and their worldwide distribution. About, 300 species of genus *Alternaria* have been identified worldwide which includes *Alternaria alternata, Alternaria tenuissima, Alternaria arborescense, Alternaria*

brassicicola, Alternaria infectoria, and *Alternaria solani* (Lee *et al.,* 2015). These pathogenic species have been reported to cause serious losses on a wide range of crops, in which *A. alternata* infects almost 100 plant species. It is also responsible for post-harvested diseases in different crops, and other diseases, including leaf spot (Rotem, 1994; Coates & Johnson, 1997; Meena *et al.,* 2017). The fungus also damages up to 30% leaf area (Srivastava, 2004). It can cause significant economic injury to a wide variety of additional hosts on which it can cause yield loss of up to 80% **(**Dudhe & Bharsakle, 2005). Some of these fungal species infect specific plants, while others have been known to infect specific plant families. Although, a wide host range has been reported for this species complex, including hosts in several plant families such as Amaranthaceae, Solanaceae, Fabaceae and Rosaceae; there is some doubt about whether the same species and strains affect all these potential hosts (Jayawardana & Hanson, 2019; Masangkay *et al.,* 2011).

They are very successful pathogenic genus that causes disease in a big number of important plants, including apple, broccoli, cauliflower, potato, tomato, citrus, pear, strawberry, tobacco, also can affect table beet and Swiss chard (Harveson *et al.,* 2010; Meena *et al.,* 2016). One commercially pertinent plant genus that can be influenced by *Alternaria* is Swiss chard as the cosmetic problem caused by symptomatic lesions can lead to rejection of crops by distributors and buyers, Host crops. Most vegetables in the Chenopodiaceae, i.e., sugar beet, table beet, and Swiss chard (Horst 2008). Number of reports has been increasing about the relative levels of resistance susceptibility to spotting on the levels. The difference between cultivars in this may be due to phenotypic and physiological characteristics such as leaf maturity behavior (Mcfarlane *et al.,* 1954; Kreis, 2016). A broad range of agricultural products can be affected with *Alternaria alternata* in many areas of the world, it was reported in Greece (Elena, 2006), Turkey, South Africa and Israel (Peever *et al*., 2002), Spain (Vicent *et al*., 2000). From this, although there is a lack or written reports, we can conclude that this fungus is most likely spread all over the world and able to proliferate in many different environments. As mentioned above, *A. alternata* expectance in several regions of the world, this fungus has been found to be responsible for different diseases (Amin *et al*., 2011).

Therefore, this study aims to identify the pathogens causing Swiss Chard disease in Tripoli using morphological criteria; also to evaluate the pathogenicity and host range. Moreover, the study conducted to determine the susceptibility of Chard cultivars to infection with *A. alternate.*

Materials and Methods:

This research was conducted to study the extent of *Alternaria alternata* disease on Swiss Chard plants. Samples of Swiss Chard plants were collected from four different sites in Tripoli; Soq – Aljumma, Al-ghrarat, Al- Harart and An-Nofleen in (2021), samples were placed in clean plastic bags, brought to the laboratory and stored at 4° c until processing within 48 hrs. The percentage of disease incidence was estimated according to (Teng & James, 2002) using the following equation:

$$\text{Percent disease incidence} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves examined}} \times 100$$

The disease intensity was assessed using rating scale from 0-5 according to (Shahzad and Bhat, 2005; Bhat *et al*., 2013), which means, (0) no leaf infections, (1) 0.1-10, (2) 10.1-25, (3) 25.1-50, (4) 50.1-75 and >75 Percent disease intensity (PDI) was calculated using formula:

$$\text{Percent disease intensity} = \Sigma (n \times v) / N \times G$$

Where, Σ=Summation; n=Number of leaves in each category; V=Numerical value of each category; N=Total Number of leaves examined; G=Maximum numerical value.

Isolation and purification of fungal pathogen

Alternaria alternata was isolated from Swiss Chard plants at the main cultivation areas in Tripoli. The disease symptoms were dark brown circular or irregular spots on infected plants. Initially, the lesions were confined within distinct parallel veins of the leaves but lately developed and convene across veins, in severe infection, the entire symptomatic leaves died.

Infected leaves were washed under running tap water to remove any contaminants before cutting it into a small pieces of about 1 cm contained half infected and half healthy portion. The small pieces of leaves were sterilized with 1% sodium hypochlorite solution for 2 minutes before rinsed with sterilized distilled water and dried on sterilized filter paper at room temperature.

The sections of infected leaves were placed on Potato Dextrose Agar (PDA), and then incubated for 7 days at 25 ± 2°C and cultures were repeatedly sub-cultured for purification. Single spore of the pathogen was isolated and maintained on PDA slants adopting Goh' s (1999) technique. The identification of the pathogen based on morphological characters; spore size, growth patterns and spore chain formation according to Ellis (1976) and Simmons (2007).

Inoculum preparation of *Alternaria alternata*.

Alternaria alternata inoculum maintained on potato dextrose agar (PDA) at 25 °C under fluorescent lights 12 hrs. per day. Cultures were transferred approximately 7-10 d before inoculation. To produce inoculum, conidia from approximately 2 weeks old, cultures were washed from the dishes, then, vortexed for 1.5 min, filtered through cheesecloth, and suspended in 200 ml sterile water with two drops Tween 20 (0.05%) as a surfactant. The spore concentration was adjusted to $5x10^5$ conidia/ml-L with the aid of a hemocytometer according to (Kohmoto *et al.*, 1991). Inoculations were performed in the early evening of January to maximize conditions for optimal infection.

Pathogenicity and Susceptibility of *Beta vulgaris* (beet) cultivars to Infection with *Alternaria alternata*.

Pathogenicity test were carried out to prove Koch postulates to confirm the ability of *Alternaria alternate,* the causal agent of Swiss Chard disease, to infect Swiss Chard plants. In this study, three -weeks-old Swiss chard transplants (certified disease-free) of five cultivars (Barese Fordhook Giant, Large White Ribbed, Lucullus and Orange Fantasia) were inoculated by spraying of 100 ml conidial suspensions of *A. alternata* concentrated (10^5/ml).

A hand atomizer was used to spray the inoculum suspensions, while control was sprayed with water. Ten plants of each cultivar were separately inoculated. Sprayed plants were maintained in a greenhouse at $25C^o$ under natural daylight conditions. The other inoculated and un-inoculated sets of test plants were placed outdoors immediately after inoculation in the early evening and exposed to prevailing natural conditions. Re – isolation of the fungi under study showing typical symptom of leaf spots of Swiss chard plants were isolated. Percentages of plant infected disease severity were determined 15 days after inoculation and calculated according to the (Shahzad & Bhat, 2005; Bhat *et al*., 2013).

This experiment was established to study the susceptibility of some cultivars to infection with *Alternaria alternata*, five cultivars of Beta vulgaris (beet) (Barese, Fordhook Giant, Large White Ribbed, Lucullus and Orange Fantasia) were evaluated by artificial inoculation under greenhouse conditions.

In this study three weeks old seedlings of Beta vulgaris (beet) were sowing in plastic pots (35 cm in diameter) filled with sterile soil sandy under greenhouse conditions, then artificially infested individually (at the rate of 1ml of suspension) with the inoculum of each tested isolate. Plants were watered and fertilized with a nutrients solutions needed.

Five replicates were used for each treatment. Fifteen days after inoculation, the incidence of disease was recorded according to (Teng and James, 2002).

Host Range.

Ten plant species belonging to six plant families grown commonly in Tripoli were tested in this experiment; Beta vulgaris (beet), *Spinacia oleracea* L., *Lactuca sativa* L. , *Brassica oleracea* , *Eruca vesicaria* L ., *Raphanus sativus* L ., *Vicia faba* L., *Pisum sativum* L., *Apium graveolens* L., *Petroselinum crispum, Solanum lycopersicum L.*. Host range experiment was conducted twice, once in the greenhouse, and again under exposed field conditions. The surface sterilized seeds / healthy seedlings of the tested host species were planted, seedlings at the two- to three-leaf stage were inoculated with 1ml of 5x 10^5conidia/mL suspension using a hand-heldatomizer prepared according to (Agnes, 2009).

Control plants were sprayed with distilled water containing 0.05% Tween 20. One inoculated set and one un inoculated set of test plants were placed in dark room with 100% relative humidity (RH) at 25°C for 14–15 h. Subsequently, pots were transferred to a "mist room," a corner of the glasshouse having 24–28°C temperature and 85–95% RH.

The other inoculated and un inoculated sets of test plants were placed outdoors immediately after inoculation in the early evening and exposed to prevailing natural conditions (in the absence of a dew supplement). Each treatment combination was replicated five times with two to four plants per pot depending on the plant species. Inoculated pots were randomly placed in greenhouse and outdoors for 15 days. (Mangala *et al.,* 2006). Plants were maintained under greenhouse and open field conditions identical to those described for the foliar pollination. Inoculated plants were examined daily for symptom development and the colour, shape and size of lesions produced on the leaves were noted. 15 days after inoculation، leaves were showed symptoms, detached and re-isolated of causal agent which performed to fulfill Koch's postulates. The percentages of plants, which showed symptoms of leaf spotting, were determined.

Results and discussion:

Survey of Swiss Chard disease in different locations in Tripoli. In this study, 100 sample of Swiss Chard were collected from the study sites in Tripoli.

Examined samples showed that 49 of them were infected with *Alternaria* leaf spot. The isolate was identified as belonging to the fungus *Alternaria alternata* on the basis of some morphological characteristics like conidial structure, presence or absence of septa, septation pattern. This is as

mentioned in relevant scientific literatures describing key morphological characteristics available for *Alternaria* (Fig. 1). Further, these isolates were confirmed on the basis of their morphological characteristics, including length of primary conidiophores, branching patterns, origin of branching, conidial shapes, size and colour with ornamentation pattern. The specie *Alternaria alternata* was identified employing compound microscope at 40X magnification with following standard manuals (Ellis, 1976; Simmons, 2007).

It can be seen in Fig.2, symptoms of disease appeared on infected plants were dark brown circular or irregular spots. The lesions were confined within distinct parallel veins of the leaves but eventually expanded and coalesced across veins. In severely affected plants, the entire symptomatic leaves died.

The spots are frequently delimited by the veins of the leaf, but infections which occur on the veins extend beyond them, on either side. Spotted leaves turned yellow and die prematurely. In the case of the leaf infections, sporulation, when it occurs, is usually on the under surface of the leaf.

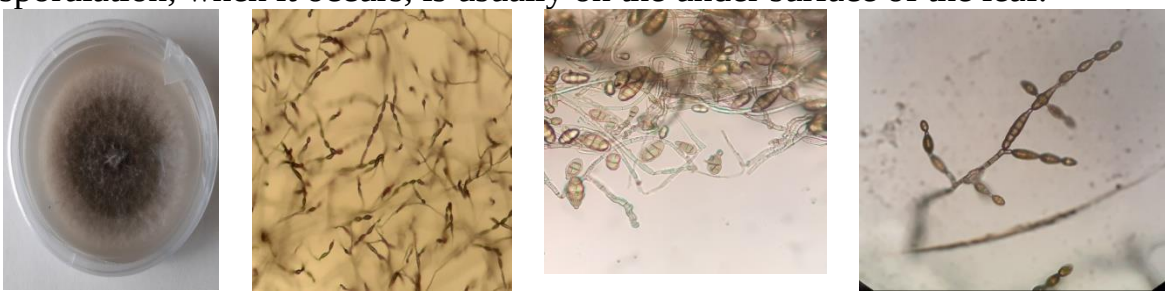

Fig. 1. Conidia and conidiophores of *A. alternata*

Fig. 2. Disease symptoms on Swiss Chard leaves.

Table 1: Susceptibility of *Beta vulgaris* (beet) cultivars to Infection with *Alternaria alternata.*

Cultivars	Greenhouse			Field		
	Disease Incidence (%).	Disease Severity	Control	Disease Incidence (%).	Disease Severity	Control
Barese	100	0.65A	0.00	90	0.29C	0.00
Fordhook Giant	90	0.55B	0.00	100	0.67A	0.00

Cultivars	Greenhouse			Field		
	Disease Incidence (%).	Disease Severity	Control	Disease Incidence (%).	Disease Severity	Control
Large White Ribbed	70	0.39C	0.00	70	0.18C	0.00
Lucullus	60	0.25D	0.00	100	0.49B	0.00
Orange Fantasia	30	0.06E	0.00	50	0.12C	0.00

The perusal of Table 1. indicates that all tested cultivars planted either in greenhouse or in the field showed varying rates in susceptibility from the results of disease incidence and disease severity. The experiment conducted in greenhouse showed that the highest percentage of disease incidence (D.I.) was 100% and 0.65 in disease severity (D.S.) for cultivar Barese, whereas the lowest was 30% in(D.I)and 0,06 (D.S.)for cultivar Orange Fantasia. However, cultivar Large White Ribbed found to be moderately susceptible, where (D.I.) was70%and (D.S.) was 0.39, compared to control.

The plant species which proved susceptible in the greenhouse were also tested in the field. The field planting was made in the spring of 2021, and infection occurred through natural means. Results are shown in Table 1. Infection took place on all plant species in the field and a little higher than in the greenhouse. The Fordhook Giant and Lucullus proved most susceptible, where (D.I.) was 100% and (D.S.) was 0.67and 0.49 respectively. But, it more slowly in the cultivar Orange Fantasia where (D.I.) was 50% and (D.S.) was 0.12, in this test.

Table 2: Host Range of *Alternaria alternata* on different hosts.

Host	Greenhouse			Filed		
	Disease Incidence (%).	Disease Severity	Control	Disease Incidence (%).	Disease Severity	Control
Swiss chard	100	0.46 A	0.00	100	0.64 A	0.00
Spinach	100	0.20 CD	0.00	70	0.30 DE	0.00
Lettuce	100	0.22 C	0.00	100	0.36C	0.00
Cabbage	100	0.11 E	0.00	100	0.33 DC	0.00
Rocket	100	0.20 CD	0.00	100	0.44 B	0.00
Radish	100	0.45 A	0.00	100	0.46 B	0.00
Faba bean	100	0.13 DE	0.00	60	0.25 E	0.00
Celery	70	0.03 EG	0.00	40	0.18 F	0.00
Parsley	100	0.01 G	0.00	30	0.16 F	0.00
Tomato	100	0.31 B	0.00	80	0.31 DC	0.00

Host range of the pathogen was tested by artificially inoculation under favorable greenhouse conditions. In this experiment, ten plants belonging to

different families were tested; Swiss Chard, Spinach, Lettuce, Cabbage, Rocket, Radish, Faba bean, Parsley and Tomato. Results showed that all tested plants were highly susceptible in the green house, but infection was classed as only moderately in the host of Celery, as shown in the results of Table 2,where (D.I.) between 70% up to100% and (D.S.) ranging from 0.01up to 0.45 compared to control.

The host range were tested under field conditions, the results showed that all tested plants were hosts to the pathogen with different (DI %) ranging from 30 – 100 presented in Table 2, while, the (DS %) was ranging from 0.16-.64) compared to control.

In green house experiment, the humidity was maintained at a high level all times and the leaf tissue became water soaked on most tested plants. This condition may have tended to predispose the greenhouse-tested plants to infection condition (Mcfarlane *et al*., 1954). In addition to that, the field conditions were also favorable for *Alternaria* infection and other fungi such as rust disease. The minor difference in the infection may be due to the deference in environmental condition and to the dispersal method of the fungi, which was air born as demonstrated by (Garg & Singh, 2016). By which, A.*alternata* was active in all months of the experiment which explain that the fungus can cause infection in high or low temperatures, that is why, infect a host range of plants from different families. Also, *Alternaria* spores are one of the most common and potent indoor and outdoor air-born allergins (Vinnewold *et al*., 1999) and (Scott, 2001) who reported that *A. alternata* is a frequently occurring species in field and storage. It produces a number of mycotoxins, of which alternariol (AOH), alternariol methyl ether (AME), altenuene (ALT) are more important and are toxigenic.

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