Journal homepage www.jzs.univsul.edu.iq
Journal of Zankoy Sulaimani
Part-A- (Pure and Applied Sciences)

Diagnosis And *In Vitro* Control Of Tomato Leaf Spot Caused By *Alternaria alternata* In Sulaimani Governorate

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Article info

Original: 31/12/2017
Revised: 19/02/2018
Accepted: 21/02/2018
Published online:

Key Words:

Tomato leaf spot, *Alternaria alternata,* Plant extracts, Salicylic acid, Tabsin-Top70

Abstract

Tomato leaf spot and stem canker is a serious disease in Sulaimani Governorate, and causes significant losses in tomato plant, this study carried out at two different locations well-known for tomato production belong to Sulaimani governorate namely; Bainjan located at Bazian district and Kanimanga at Penjween district for this purpose 3 fields at each location randomly taken to study the incidence, severity of the disease, identification the isolates and using 4 different plant extracts namely, (*Hypericum triquetrifolium* L, *Punica granatum* L, *Allium sativum* L and *Thymus vulgaris* L) for their antifungal activities against A. alternate. The results detected that the highest value of the disease incidences of Alternaria leaf spot of tomato in the two locations were 74.65, 73.19% in Bainjan and Kanimanga respectively, and the maximum disease severities were recorded 78.06 and 67.70% in Kanimanga and Bainjan fields respectively. The data from this fungus, and its isolates indicated that there were two different isolates, each isolate has specific morphological characteristics in their mycelial growth and also different in conidia measurement and their beaks and the number of cells in each conidium. The mean length and width of the Ka isolate conidia were 31.93, 12.36 µm and the mean of beak was 6.52, 4.15 µm for length and width respectively while the mean of Ba isolates was reached 27.68, 9.84 µm for length and width respectively, the mean of conidia beaks was 4.04, 9.84 µm for width and length respectively. Regards to the number of cell, transverse and longitudinal septa Ka for another time recorded superior against Ba isolate. *P. granatum* extract was revealed the highest mycelial growth inhibition (MGI 100%) for both isolates when used at 80000 µg ml^{-1}, and the next was *H. triquetrifolium* also recorded maximum (MGI 100%) for Ba isolate and (93.08%) for Ka isolate, when used at 8%. Aquatic extracts of *A. sativum* significantly inhibited the fungus growth (89.44%), (83.02%) for Ka and Ba isolates respectively at 30%. *T. vulgaris* comes next in fungus inhibition efficacy beyond *A. sativum* and recorded (80.58, 78.11%) for Ka and Ba isolates respectively at 30%, 50 ppm of Salicylic acid and Tabsin-Top70 proved minimum mycelial inhibition rate and recorded (23.02 and 45.11%) for Ba, and (41.14 and 22.38%) for Ka isolate at the same concentration. Meanwhile, the MGI reached to 100%when salicylic acid used at 400ppm for both isolates. The MGI were 84.64 and 89.11% when used Tabsin-Top70 at 600ppm for Ka and Ba isolates respectively.

Introduction

Tomato *Alternaria* leaf spot and stem canker caused by *Alternaria alternata* pathotype was first reported in San Diego, the USA in 1960 and later found worldwide. The fungus overwinters in infected plant debris, in/or on the soil where it can survive at least one and perhaps several years *[1]*. The genus *Alternaria* have many species of worldwide distribution. The species of this genus are considered to be infectious and polyphagous fungi, in the absence of the specific host they easily grow on dead and decaying organic materials and is responsible for causing leaf spot diseases in a number of economically important crop plants. Cerkauskas (2005) *[2]*; Momel and Pemezny (2006) *[3]* supported that this disease causes a great reduction in the quantity and quality of fruit yield. In addition, the disease is favored by warm temperature and extended periods of leaf wetness from dew, rainfall, and crowded plantation, the plants are more susceptible to infection by the disease during fruiting period. *Alternaria* produces a wide range of symptoms at all stages of plant growth. The disease appears on leaves, stems, petiole, twigs and fruits under favorable conditions resulting in defoliation, drying off of the twigs and resulted in premature fruit drop *[4]*. In disease management strategy, various methods have been used. The natural plant products, known as botanical pesticides, have long been used in the control of this disease. Several workers have reviewed the various developments in the biological control of the plant pathogen *[5]*. Salicylic acid (SA) represents an interesting new opportunity in controlling fungal diseases within an environmental friendly integrated crop protection system through enhancing the resistance of the plant to the pathogen and inhibiting the growth of various pathogen *[6]*. Plant extracts from the various parts of certain plant species have been successfully tested to demonstrate their antifungal activities *[7]*. Because of the absence of or rare works on this serious disease in Kurdistan especially in Sulaimani Governorate, therefore this study was conducted to detect and identify of causal pathogen(s) from two locations (Bainjan in Bazizn district, and Kanimanga in Penjween district and to evaluate the efficacy of some plant extracts and chemicals *in vitro* efficiency.

Materials and Methods

A. Field sampling and disease assessing

For assessing the incidence and severity of tomato leaf spot disease two locations were selected, which were Bainjan of Bazian district and Kanimanga of Penjween district. At each location three fields were randomly determined and for this purpose from each field, 5 random rows were selected. In order to estimate the disease severity 0-5 disease evaluation scale was used as described by *[8]*, while 0 is no symptoms, 1: 1-5 %, 2: 6-20%, 3: 21-40, 4: 41-70 %.and 5: >71 % leaf area infected and covered by the spots, and spots seen on branches, stems and fruits.

For estimating the disease incidence *[9]* and disease severity *[10]*, the following formula was used and*:*
Disease incidence (%)= No. of infected plants / No. of total plants X 100, and, Disease severity (%) = Sum of the individual disease ratings / Total No. of diseased plants x Maximum disease grade X 100.

B. Isolation and identification of the causal pathogen(s)

From tomato leaf spots infected with pathogen (s) was isolated from tomato leaves and other parts (stem and fruits), with visible symptoms on the infected parts of the plant. The plant pieces (5 mm) were thoroughly washed, then were cut, superficially sterilized in sodium hypochlorite solution (1%) for 4-5 min *[11]*, the specimens were placed on PDA medium (200g potato, 15g Dextrose ,15g Agar

and 1L distil water), incubated at (25± 2°C) for 7 days, and purified using hypha-tip isolation technique and/or single-spore isolation techniques *[12]*. Based on the morphological characteristics, the pathogen(s) were studied *in vitro* for identification depending on the colony growth and the structures of conidia and then measured using adopting slide culture technique with the aid of (AmScope, 12MP USB 2.0 Real-time live video Microscope Digital Camera, Model MU500, China), which was adapting with microscope to study the conidiophores formation, structures of conidia and their colors, the beaks of conidia, the transverse and longitudinal septa as well as the length and width, then the pathogen was identified *[13]*. Finally, for the confirmation of identification of the isolated pathogens, pure samples were sent to the Collage of Science/Department of Biology/University of Salahaddin, Assist Prof. Fareed Matti Toma.

C. Pathogenicity test

The pathogenicity test was carried out under plastic house conditions. Tomato seeds (cv. Kurdish Sangaw) were sown in stiropor trays 84 (7 x 12) cells (purchased from local market, unknown company) with sterilized compost. After 25 days from sowing the seedlings transplanted into plastic pots (15 cm) which, were contained 2kg of (1:1) sterilized soil/compost. For inoculation purpose 7 days old pure cultures were used to prove pathogenicity of the fungus on tomato plants. Spore suspension was prepared from 10 ml of sterilized, distilled water added to the pure culture and the conidia were collected using a sterilized brush, then the spore suspension was filtered through three layers of Muslin cloth. The conidia suspension was adjusted to (4×10^6 CFU ml^{-1}) using Hemocytometer (Burker, Marienfield. Germany). After 2 weeks from transplanting, when the plants were 40-45 days old, the plants were sprayed with the purified spore suspension of both isolated fungi (Ba and Ka) with the aid of the hand atomizer, then maintained in the plastic house (25-27 °C). The inoculated plants were sprayed with atomized water to obtain moisty conditions with humidity of more than 85% with different wetness periods (12, 24, 48 and 72 hrs.) this was achieved by locating the plants in different covered chambers, other pots maintained in dry condition without spraying with water, inoculating and covering as control treatment. Each treatment is replicated 5 times (5 plants). Daily observations were taken to monitor the development of disease symptoms on leaves and stems, after 2-3 weeks of the process, the symptoms appeared on inoculated leaves. Re-isolation and purification culture from these artificially infected plants were conducted.

D. Collection of plant materials

Four different mature plant species, namely (*Hypericum triquetrifolium* L, *Punica granatum* L, *Allium sativum* L, *Thymus vulgaris* L) were selected and tested for their antifungal activities against *A. alternata*. Samples of wild and domestic plants were collected from different locations of Sulaimani governorate. The healthy plant parts were washed thoroughly 2-3 times with running tap water. The plant parts (leaves, stems, and peels) were air dried at room temperature (20-25°C) for three weeks in the shade conditions, the dried samples were then ground to powder by an electric grinder, and kept in plastic containers under a dried condition for extraction.

E. Plant extracts

Two methods were followed to obtain plant extracts, etanolic and aquatic. In the ethanolic extraction, whole plant (except roots) of *H. triquetrifolium* were conducted according to, Pomegranate peel was homogenized and extracted according to *[14]*, the two gummy extracts were kept in the

refrigerator (4°C) until using after that 1, 2, 4, 6, and 8% concentration were used. Fresh garlic (*Allium sativum* L.) cloves were peeled and surface sterilized using 1% sodium hypochlorite solution for two minutes and washed by sterile distill water thrice, materials were prepared and homogenized according to *[14]*. For the two plants, aquatic extracts (1, 2.5,5,10,20 and 30%), were prepared *[15]*.

F. In vitro testing of plant extracts

Poison Food Technique (PFT) was used to determine the antifungal activities of the plant extracts. *[17]* Five concentrations of the gummy extract of *H. triquetrifolium* and *P. granatum* were prepared (1, 2, 4, 6 and 8 ml/100 ml medium equal five concentrations 10000, 20000, 400000, 60000 and 80000 µg ml^{-1} each). Subsequently, 5 mm diameter mycelial disks were taken from 7 days old pure culture of *A. alternata* using sterile cork borer and the petri dishes were inoculated at their centers in which the discs were placed up down. Other Petri dishes were kept without plant extracts as a control. The inoculated plates were incubated at 25 ± 2°C. The experiment was arranged in completely randomized design (CRD) and replicated four times. The growth of the fungus was monitored daily until the fungus in control plate reached the edge of the border of the Petri plates, the radial growth of the pathogen was measured using ruler at two different angles at 90° to each other and the mean calculated *[18]*. Fungal-toxicity of tested extracts was calculated in terms of percentage growth inhibition according to the following formula *[16]*.

PGI (%)= DC – DT / DC x DC

PGI=Percentage of growth inhibition, DC= Average growth diameter of fungal colonies obtained from control plates, DT = Average growth diameter of fungal colonies obtained from treated plates.

G. In vitro evaluation of the fungicide (Tabin-TopM70) and Salicylic acid

Poison food technique (PFT) *[19]* was followed to evaluate the efficacy of different concentrations of the chemicals (50, 100, 200, 300, 400, 500 and 600 ppm) of the fungicide Tabsen-TopM70 (Thiophanate-methyl 70%, We-Young company, China). Salicylic acid (with purity of 99.8%, Soyoung Biotechnology company, China), with concentrations (50, 100, 200, 300, 400 and 500ppm) were tested *in vitro* for *A. alternata.* Molten sterilized PDA was used as a nutrient medium and fungicidal suspensions of different concentrations were prepared by dissolving requisite quantities of each chemical in warm media at 50 °C before pouring and shacked well. About 15 ml of poisoned medium was poured in each 9cm sterilized Petri dish. 5 mm discs of 7 days old cultures of fungal isolates were placed in each petri dish. Numbers of Petri dishes were kept with the chemical as a control. Colony growth was measured in (cm). Percent inhibition of radial growth was calculated, the following formula was used *[17]*.

Growth inhibition (%) = Growth in control – Growth in treatment / Growth in control x 1

H. Statistical Analysis

The data have been analyzed statistically by using (XLSTAT) computer program version (7.5.2). Means were compared according to Duncan's Multiple Range Test (DMRT) at ($p \leq 0.05$).

Results and discussion

A. Disease incidence and severity

Table (1) shows the disease incidences and severities for both isolated fungi, the means of disease incidences were 74.65 and 73.19%, and the means of disease severities were 67.70 and 78.06% for

Bainjan and Kanimanga respectively. There are no significant differences of both locations in the disease incidence despite of each location has the specific environmental condition (air temperature and relative humidity), but for the disease severities there were significant differences between those two locations, this might be due to the differentiation of relative humidity and temperature. The distance between the two locations is more than 160 km, thus made the variety of disease incidence and severity because of differences in environmental condition. A similar result has been pointed to when the environmental conditions are favorable (relative humidity and temperature), the pathogen (*A. alternata*) increases plant ability for infection *[4]*. Another investigator referred to the serious damage to the crop when the conditions were favorable *[20]*.

Table-1: Disease incidence and severity of Tomato Leaf Spot caused by *A. alternata* in two different locations**.**

Location name	*Mean of total plants **	*No. of Infected plants*	*Disease incidence %*	*Disease severity %*
Bainjan field 1	*24.00*	*17.20*	*71.25*	*74.83*
Bainjan fiield 2	*25.40*	*19.20*	*75.52*	*85.53*
Bainjan field 3	*19.00*	*13.80*	*72.80*	*73.83*
Mean	*22.80*	*16.73*	*73.19*	*78.06*
Kanimanga field 1	*16.20*	*12.00*	*73.89*	*65.75*
Kanimanga field 2	*12.60*	*9.40*	*73.92*	*69.07*
Kanimanga field 3	*20.80*	*16.00*	*76.14*	*68.29*
Mean	*16.53*	*12.47*	*74.65*	*67.70*

*Each number representing the means of five rows

B. Morphological studies of tomato leaf spot causal pathogen

The color of colonies grown on PDA after 7 days of incubation was gray to sooty-black and became grayish black with white margin and later covered with dark green plush appearance with the age of colony advancing (Fig 1), similar finding was conducted by *[2]* and *[24]*. Kumer *et al*. (1985) *[25]* recorded that the fungus was recognized by the presence of olivaceous black or black colonies. Blanchard, 2012 *[23]* described that the colonies of the fungus as initially light grey later turning black to olivaceous black. On the basis on morphological study the fungus was identified as *Alternaria alternata*. Also, the variation in the morphological status of isolates (the conidia length, width, color and the length, width of conidial beaks) of the two location (Bainjan, Kanimanga) lead to determine and naming two different isolates (Ba and Ka) *[26]*.

Figure-1: Front and revers side of *Alternaria alternata* grown on PDA.

Total microscopic features of the studied fungus, illustrated in (Fig 2, 3). The conidiophores appeared as curve shape, varied in length, and simple or branched and light - brown to olive-brown in color, the current results agree with the previous findings of *[21]*. Size of conidial spores was based on measurements of 30 randomized conidia. The conidia of both two culture locations (Ba. and Ka.) were obclavate, brown to dark olive. Conidia of Ba isolate with 3 to 4 transverse septa and 1 to 2 longitudinal and/or oblique septa. The average of length and width were 27.68, 9.84 µm respectively, while the length and width of the conidia beak different in size with 4.04 and 3.89 µm respectively.

In Ka isolates the conidia had 3 to 5 transverse and 2 to 3 longitudinal septa. The average lengths were 31.93 µm and 12.36 µm in width, also the measurement of conidia beaks calculated and recorded 6.52 and 4.15 µm for length and width respectively. The longest conidia were recorded in Ka isolate 41.49 µm, while the shortest ones recorded in Bai isolate (21.81 µm). Conidia of *A. alternata* have short beaks, compared to *A. solani*, this point is considered to be the distinguishing diagnostic factor of the two Alternaria species, and with differentiation of both transverse and longitudinal septa *[26]*. Conidia range from 20 to 60 µm by 9-18 µm dimension, and their color is greenish brown, obclavate and may be straight to geniculate *[27]*. The distance between the two districts more than 160 km, thus made the isolates of the *Alternaria*, which lead to some differential variation; such as septa, measurements of length and width of beaks and number of cell/conidia. In respect of this, Abobakar and Ado (2009) *[28]* observed the same results and agreement of the effect of variation in environmental factor on the variation of shape and dimensions of the *A. alternata* isolates.

Table-2: Morphological characteristics of 30 conidia and conidiophores of *A. alternata* were randomly taken from two locations in Sulaimani Governorate.

Morphological characteristics	*Bainjan isolate*		*Kanimanga isolate*	
	Range	*Average*	*Range*	*Average*
Conidia length (µm)	*21.81-36.05*	*27.68*	*22.39-41.49*	*31.93*
Conidia width (µm)	*7.62-13.28*	*9.84*	*9.06-14.79*	*12.36*
Length of conidia Beaks (µm)	*2.69-5.84*	*4.04*	*4.13-7.92*	*6.52*
Width of conidia Beaks (µm)	*3.30-4.87*	*3.89*	*2.82-6.88*	*4.15*
Transverse septa	*3–4*	*3.73*	*3–5*	*4.1*
Longitudinal septa	*1–2*	*1.4*	*2–3*	*2.06*
No. of cells / conidium	*5–7*	*6.1*	*5–8*	*7.16*
Colony color	*Dark green*		*Dark green*	
Conidiophore	*Septate, olive brown and branched*		*septate, olive brown and single or branched*	

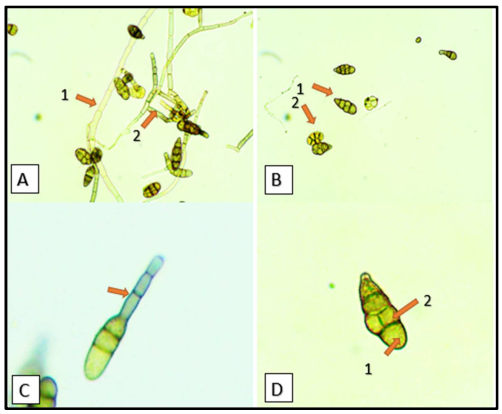

Figure-2: (A-E) Morphological characteristics of *A. alternata* (Bainjan isolate) of conidia and conidiophore. A. Conidiophore and their conidia (10x40), B. (1) Beaked and (2) un-beaked conidia (10x40), C. (1) Transverse and (2) longitudinal septa (10x100), D. Germ tube elongation (10x100)

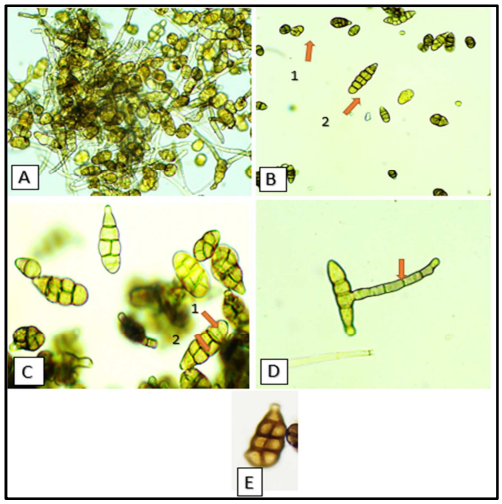

Figure-3: (A-E) Morphological characteristics of *A. alternata* (Kanimanga isolate) of conidia and conidiophore. A. Conidiophore and their conidia (10x40), B. (1) Beaked and (2) un-beaked conidia (10x40), C. (1) Transverse and (2) longitudinal septa (10x100), D. Germ tube elongation (10x100), and E. Conidia with number of cells (10x100)

C. Pathogenicity tests

Results shown in (Fig. 4 A, B) revealed no any symptoms (Alternaria leaf spot) appeared on the plants in both uncovered plants, this means that the disease incidences (DI) and disease severities (DS) were 0% for both (Ba and Ka isolates) respectively. This observation similar to the work of another researcher, who refer to no disease symptoms on tomato plants in the absence of leaf wetness, while inoculated with *A. alternata [29].*

The (fig 4, A) showed that raising the wetness period from 12hrs (DI and DS were 20%) to 24 hrs. the DI and DS reached to 100 and 66.66% respectively, regarding to Bainjan isolate, also *[30],* they referred to similar results found that a minimum dew period of more than 6 hrs. was required for lesion development.

From (fig. 4, B) the data indicated that the wetness period at 12hrs, the DI and DS were 40%, while when wetness period is raised to 24 hrs. the both DI and DS were increased to 100% and 60% each. Similar results were recorded for both isolates when the wetness periods reached to 48 and 72 hrs. the DI and DS were 80 and 85% in Bainjan and Kanimanga respectively. Based on the results of the present study, the disease incidence and severity were directly relative to the wetness of the plant leaves, Other researchers reported similar results for other *Alternaria* spp. *[31]* and *[32]*. Balai and Ahir (2013) *[33]* also observed maximum MGI% of *Alternaria* sp. at 100% relative humidity. Pathogenicity test revealed that the two isolates were pathogenic and virulent. Three to four weeks after inoculations the symptoms appeared on inoculated leaves as small brown, round to oval necrotic spots. Re-isolated and purified culture from these artificially infected leaves were similar to original culture. This result agrees with the finding of *[34]* who also proved the pathogenicity in case of *A. alternata* were compared and similar with original culture.

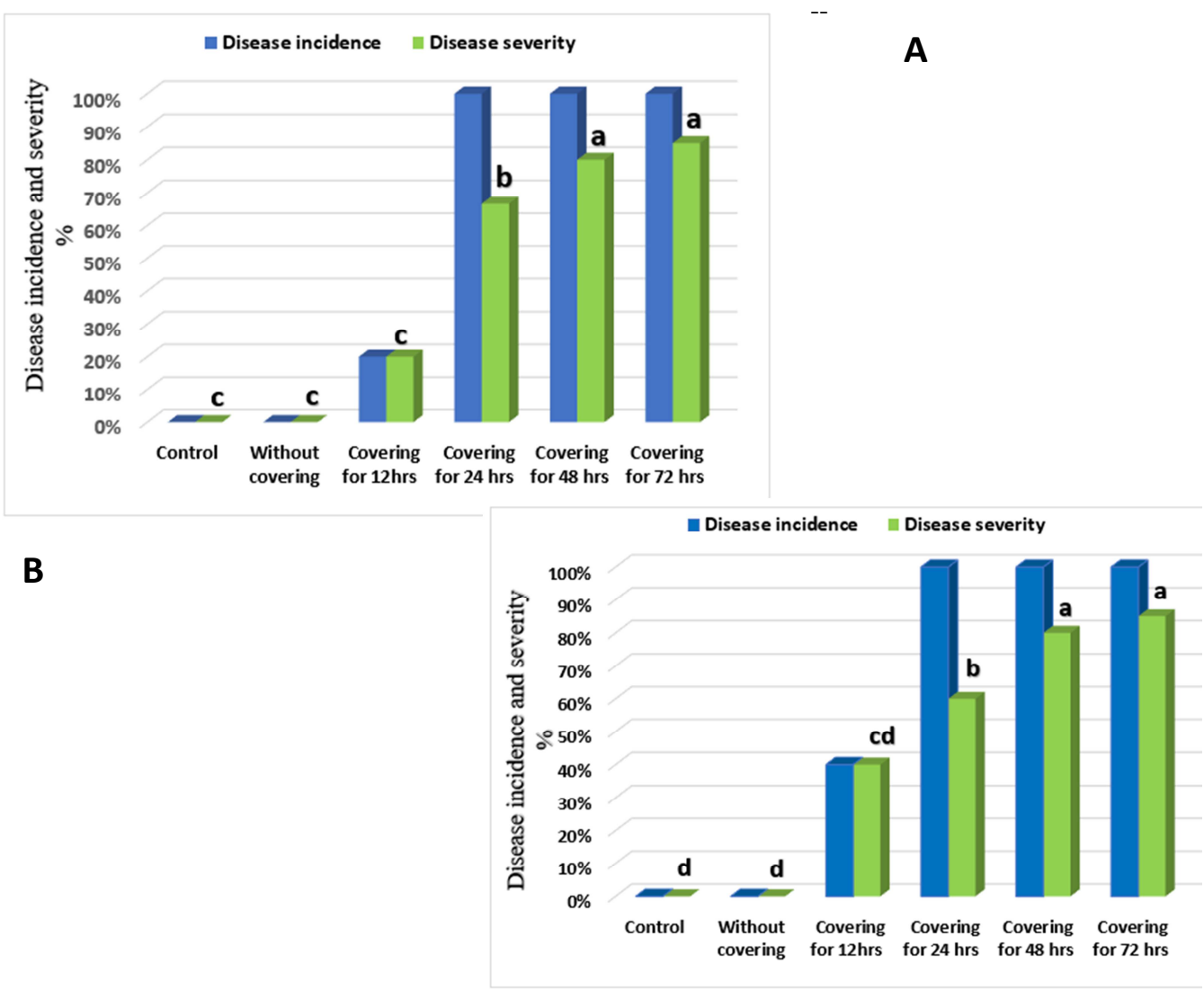

Figure-4: Effect of wetness duration on pathogenicity of *A. alternata* A. (Bainjan isolate) B. (Kanimanga isolate)

D. In vitro antifungal activities of plant extracts

It was revealed from the results of Table (3) that different concentrations of ethanolic plant extracts caused significant influences on inhibition of mycelial growth of the fungus. However, the maximum inhibition of the mycelial growth was occurred, when used at highest concentration (10000 µg ml^{-1}) of *Punica granatum* (100%) for both isolates of the fungus, while the MGI% was recorded (53.14%) at (10000 µg ml^{-1}), that's mean that the inhibitory concentration (IC_{50}) of this crude extract starts from (10000 µg ml^{-1}) in Ba. isolate. For Ka. isolate, the minimum was (49.05%) at (10000 µg ml^{-1}) means the (IC_{50}) also starts from (10000 µg ml^{-1}).Some researchers referred to similar studies investigated the *in vitro* effect of ethanolic crude extract of *P. granatum* on *A. alternata, F. oxysporum, Phoma destructiva, R. solani,* and *S. rolfsii* at different concentrations, also the efficacy

of pomegranate peel extract (PPE) against pathogenic fungi *Mycosphaerella arachidicola*, *F. oxysporum*, *Helminthosporium maydis*, *Valsa mali* and *R. solani* have been investigated by *[35].* It has been known from the following researchers that the (a.i.) of Pomegranate peel were tannins and their derivatives, which affected on the *A. alternata* isolated from the both Ba and Ka regions.

Table (3) demonstrate the effect of different concentrations of *H. triqutrifolium* on the mycelial growth of both *A. alternata* isolates, the (MGI) was 100% was recorded for (80000 µg ml^{-1}) in Bainjan *A. alternata* isolate, while the minimum (MGI%) was recorded for the same isolate (45.58%), and the inhibitory concentrations (IC_{50}) was between (45.58 to 63.81%) for the two concentrations (10000 to 20000 µg ml^{-1}.). In Kanimanga isolate, the maximum MGI% was 93.08% at 80000 µg ml^{-1} concent, while the minimum MGI% was 43.52% when 10000 µg ml^{-1} used and the (IC_{50}) was (53.11%) at 20000 µg ml^{-1}. Some researchers were studied the effect of ethanolic crude extracts of *Hypericum* sp. on *A. alternata* and indicated that fungus was susceptible by poisoned food technique *in vitro [36].*

Table-3: Effect of two ethanolic crude extract on mycelial growth inhibition (MGI) of *A. alternata* (Ba and Ka isolates)

Plant extract	*Concentration* (µg ml^{-1})	*Growth inhibition percent (GI %)**	
		A. alternata (Ba isolate)	*A. alternata (Ka isolate)*
Hypericum triquitrifolium	*0*	*0.00 (0.00) i*	*0.00 (0.00) k*
	10000	*45.58 (42.45) h*	*43.52 (41.28) j*
	20000	*63.81 (53.02) f*	*53.11 (46.78) h*
	40000	*70.76 (57.26) e*	*65.32 (53.94) g*
	60000	*82.11 (64.97) c*	*75.73 (60.50) e*
	80000	*100.00 (90.00) a*	*93.08 (76.73) b*
Punica granatum	*0*	*0.00 (0.00) i*	*0.00 (0.00) k*
	10000	*53.14(46.80) g*	*49.05 (44.46) i*
	20000	*69.91 (56.71) e*	*72.67 (58.48) f*
	40000	*79.47 (63.04) d*	*78.20 (62.16) d*
	60000	*85.99 (68.01) b*	*84.03 (66.04) c*
	80000	*100.00 (90.00) a*	*100.00 (90.00) a*

*Data are means of four replicates and the data between parentheses are angular transmitted
Means followed by the same letter within a column are not significantly different at ($P \leq 0.05$) according to Duncan's Multiple Range test.

E. In vitro effect of two chemicals.

Two chemicals salicylic acid (SA) and a systemic fungicide Tabsin-Top 70% were evaluated against *A. alternata in vitro.* The results indicated that there were significant differences between (SA) and Tabsin-Top in inhibiting the growth of *A. alternata*. The data referred to increasing concentrations of both chemicals caused increased inhibition of mycelial growth of the fungus.

Data presented in Tables (4) showed the fungus revealed more sensitivity against (SA) if compared with the Tabsin-Top at all concentrations used for both isolates. Moreover, the fungus was significantly inhibited by SA and highly reduced the growth of the fungus at 400, 500 ppm, and get

(100%) MGI of the two isolates Ba and Ka regions, while the minimum MGI was recorded (23.02%) and (41.41%) at 50ppm for Ba and Ka isolates respectively.

The IC_{50} for SA was between (46.97-74.11%) when used at 100-200 ppm for Ba. isolate, and (50.41%) at 100ppm for Ka. isolate. Srinivas *et al*. (1997) *[37]* indicated that SA was highly effective in inhibiting the *in vitro* mycelial growth of *Alternaria* sp. Moreover, the fungal growth was completely inhibited by salicylic acid at the concentration of 200 ppm, also observed that the lowest IC_{50} for 50% growth inhibition values were achieved by salicylic acid (78.2 ppm). The highest inhibition of the fungus *A. alternata* (Ba isolate) has occurred when Tobsin-Top70 at 600ppm which achieved 89.11% since the minimum MGI for the same isolate was 45.11% at 50 ppm. The maximum MGI was 84.64% at 600 ppm and the minimum MGI was 22.38% for (Ka isolate) by using 50 ppm. The data referred to increasing concentrations of both chemicals caused increased inhibition of mycelial growth of the fungus. The IC_{50} for Tabsin-Top 70% ranged between (100-200ppm) were recorded 46.44-54.41% for Ba isolate and 49.94% for Ka isolate when used at 300 ppm *[38]*.

Table - 4: Effect of two chemicals on mycelial growth inhibition (MGI) of *A. alternata* (Ba and Ka isolates).

Chemicals	*Concentration ppm*	*Growth inhibition percent (GI %)**	
		A. alternata (Ba isolate)	*A. alternata (Ka isolate)*
Tabsin Top M70 WP	*0*	*0.00 (0.00) l*	*0.00 (0.00) k*
	50	*45.11 (42.20) k*	*22.38 (28.23) j*
	100	*46.44 (42.93) i*	*30.29 (33.38) i*
	200	*54.41 (47.51) h*	*37.61 (37.83) h*
	300	*65.29 (53.89 j*	*49.94 (44.95) f*
	400	*70.29 (56.97) f*	*55.79 (48.33) e*
	500	*79.44 (63.04 c*	*73.70 (59.14) d*
	600	*89.11 70.58) b*	*84.64 (66.93) b*
Salicylic acid	*0*	*0.00 (0.00) i*	*0.00 (0.00) k*
	50	*23.0 (28.67) k*	*41.41 (40.06) g*
	100	*46.97 (43.26) i*	*50.41 (45.22) f*
	200	*74.11 (59.40) e*	*73.41 (59.10) d*
	300	*75.76 (60.52) d*	*79.79 (63.29) c*
	400	*100.00 (90.00) a*	*100.00 (90.00) a*
	500	*100.00 (90.00) a*	*100.00 (90.00) a*

*Data are means of four replicates and the data between parentheses are angular transmitted
Means followed by the same letter within a column are not significantly different at ($P \leq 0.05$) according to Duncan's Multiple Range test.

F. In vitro antifungal activities of aquatic plant extracts

Table (5) demonstrate the evaluations of the inhibitory effect of *A. sativum* and *T. vulgaris* on *A. alternata* (Ka and Ba isolates). The aquatic extracts of *A. sativum* significantly achieved maximum mycelial growth inhibition 83.02 and 89.44% of the fungus *A. alternata* at conc. 30% for both isolates at the two locations Bainjan and Kanimanga respectively, while the minimum MGI% for *A. sativum* extract revealed 20.38 and 35.70% at 1% conc.

for Ba and Ka isolates respectively. The IC_{50} aquatic extract of *A. sativum* was 5% were recorded 50.64% inhibition of the fungus for (Ba isolate), since the IC_{50} was 5-10% of the same extract, the inhibition rates were between 47.82-65.91% for Ka isolate.

Some researchers referred to the efficacy of the aquatic extract of *A. sativum* on MGI% of *A. alternata [39].* Daniel *et al*. (2015) *[40]* also investigated that aqueous dilutions of the garlic extract showed significant activity than the ethanol dilutions of the extract. Antifungal properties of garlic extracts have also been observed against the growth of *A. alternata, [41].*

On the other hand, *T. vulgaris* comes next after *A. sativum* as anti-fungal plant extract for both isolated fungi, maximum MGI of *T. vulgaris* aquatic extract was (80.58%) at 30% conc. and the minimum value of MGI was recorded 31.08% at 1% concentration for (Ka isolate), for (Ba isolate) also the maximum value of MGI was recorded less than previous data 78.11% at 30% concentration and the minimum was 34.52% for 1% conc. The IC_{50} for *T. vulgaris* was between 5-10% was (46.51.58.29%) for Ba isolate and the IC_{50} for the same extract was 5% (52.79%) for Ka isolate. Many reports pointed to the similar studies, that *T. vulgaris* has antifungal activity *[42].*

Table-5: Effect of some plant aquatic extract on mycelial growth inhibition (MGI) of *A. alternata* (Ba and Ka isolates)

Plant extract	*Concentration %*	*Growth inhibition percent (GI %)**	
		A. alternata (Ba isolate)	*A. alternata (Ka isolate)*
Allium sativum	*0*	*0.00 (0.00) m*	*0.00 (0.00) m*
	1	*20.38 (26.83) l*	*35.70 (36.69) j*
	2.5	*33.73 (35.51) k*	*44.61 (41.90) i*
	5	*50.64 (45.35) g*	*47.82 (43.73) h*
	10	*54.41 (47.52) f*	*65.91 (54.27) e*
	20	*66.91 (54.89) d*	*77.97 (61.84) c*
	30	*83.02 (65.68) a*	*89.44 (71.02) a*
Thymus vulgaris	*0*	*0.00 (0.00) m*	*0.00 (0.00) m*
	1	*34.52 (35.97) j*	*31.08 (33.89) l*
	2.5	*40.23 (39.36) i*	*33.35 (35.27) k*
	5	*46.61 (43.03) h*	*52.79 (46.59) g*
	10	*58.29 (49.76) e*	*57.61 (49.37) f*
	20	*67.38 (55.16) c*	*72.67 (58.46) d*
	30	*78.11 (62.11) b*	*80.58 (63.85) b*

*Data are means of four replicates and the data between parentheses are angular transmitted

Means followed by the same letter within a column are not significantly different at ($P \leq 0.05$) according to Duncan's Multiple Range test.

Conclusions

According to the results obtained from this study concluded that samples of tomato leaves and stems, which were collected from the two locations (Bainjan and Kanimanga) and infected with leaf spot and stem canker indicated that the causal pathogen identified as *A. alternata* with two different isolates. From studied samples, *A. alternata* showed significant disease severity, damage and loss of tomato

fields indicated that the fungus economically important. The results showed that the two isolates were variance in their morphological characteristics and their responses to the plant extracts and fungicides, also it was revealed that the plant extract significantly more effective than the chemicals, thus it was advised using natural plant extracts, because of their safety to human beings and environment and for their cheap costs and availability. Using Salicylic acid as a fungicide for controlling the fungus revealed that it was with significant effect, and reduced the mycelial growth inhibition rate (MGI) to a high level reached to 100% and this chemical is safe and cheap, this is the first attempt, formerly has not been recorded in the literature. We consider that this work is the first scientific paper about diagnosing the causal pathogen and declaring the importance of *Alternaria* leaf spot and its controlling in Sulaimani Governorate.

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