Egypt. J. Chem. **Vol. 66**, No. 9 pp. 421 - 438 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Biological control and molecular differences among some isolates of *Alternaria solani*, the causative agent of early blight in potatoes

Mohamed A. M. Abdel-Hamid *, M. A. Khalil **, Mohamed S. Abbas *, Abd-El-Moneim-Maisa L. * and Amira Sh. Soliman *****

** Central Lab., Org. Agric., Agric. Res. Centre, Giza, Egypt.*

*** Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.*

**** Department of Natural Resources, Faculty of African Postgraduate Studies, Cairo University, Giza, Egypt.*

Abstract

In this study, some isolates of Alternariasolani; the causative agent of potato early blight disease were isolated and identified from different Egyptian governorates. The pathogenicity of these isolates was examined on the potato cultivar (Lady-Rosetta). The genetic variation among these isolates were identified using the RAPD and SDS-PAGE techniques. Also, the antifungal activity of some biological agents against the highest aggressive isolate of El-Minia governorate (El-Borgia No.1) was evaluated in the laboratory, the greenhouse and the field. Five isolates were isolated from leaves and tubers of naturally infected potato plants, collected from fields planted with the varieties (Kara, Lady-Rosetta and Spunta) representing different localities of some governorates. The fungal isolates were symbolized from 1 to 5 according to their regions. Data showed that all tested isolates proved to be pathogenic to the tested potato (cv. Lady-Rosetta) causing symptoms of early blight comparing with the control. The fungal isolate No. 1 which was obtained from (El-Borgia, Minia governorate) was the most aggressive isolate with the highest significant values of disease severity (44.30 and 56.19%) of the two successive seasons, respectively. Regarding to, RAPD-PCR, SDS-PAGE protein patterns and phylogenetic analysis data showed that isolates 1, 2 and 3 that have the high pathogenicity were similar to each other and share the same cluster while the other fungal isolates 4 and 5 with the low pathogenicity were phylogenetically close to each other. Moreover, there were no relationship between the pathogenicity and the geographic distribution of all tested A. solani isolates. Finally, in vitro antagonistic test declared that Trichodermaharzianum was the best bioagent that gave the highest inhibition zone (67.33cm) against the most aggressive A. solani isolates as compared with all other bioagents. Spraying the sensitive potato plants (cv. Lady Rosetta) in the greenhouse and field antagonistic with T. harzianum suspension showed the best results and gave the lowest values of early blight disease severity during the two seasons (19.77, 11.25%) and (25.32 and 10.66%, respectively) as compared with the control and other treatments.

Keywords: Potato, Alternariasolani, Pathogenicity, SDS-PAGE, RAPD-PCR, Biological control.

*Corresponding author e-mail: m66583460@gmail.com (Mohamed A. M. Abdel-Hamid)

Received date 2022-12-08; revised date 2023-01-08; accepted date 2023-01-09
DOI: 10.21608/EJCHEM.2023.179624.7289

Introduction

Potato (*Solanumtuberosum*L.) is one of the most important crops in Egypt as well as all over the world and ranks the world's fourth most paramount food crop after maize, wheat and rice. It produces tubers very rich in starch, which is considered a source of low-cost energy to human diet and a rich source of vitamins especially Vit C and Vit B1. It contains different minerals,20.6 % of carbohydrates, 2.1 % of protein, 0.3 % of fats, 1.1 % of crude fibers and 0.9 % of ash. It contains a good amount of essential amino acids like leucine, tryptophan and isoleucine …etc. as well **[14]**.

Under Egyptian climate conditions, early blight disease caused by *Alternariasolani*, is an important disease of potatoes. The disease can cause substantial yield losses if it was not appropriately controlled **[2]**.Symptomatic lesions of early blight are characterized by dark concentric circles that are restricted within leaf veins. Diseases distribution differs according to the sowing date (seasons), *i.e.* summer (January), nili or fall (September-October) and winter (November-December) plantations. Area in Minia governorate under potato plants cultivation is increased year by year, thus intensive cultivations of potato plant led to infection of numerous pests and diseases, which resulted in crop losses and increased production costs **[47] and [20]**. Developing new potato cultivars with resistance to early blight disease may reduce losses in the field,
storage and lessen the need for fungicides application**[10]**.

RAPD-PCR (polymerase chain reaction) analysis is commonly utilized to investigate genetic variation in populations of *Alternaria*species in regard to the cost efficiency, rapid output and ability to detect polymorphisms in specific gene fragments **[24]; [26]; [35]; [46]and [42]**.

Gel electrophoresis of proteins has been widely used for studying variation in fungal populations. SDS-PAGE method is used because it alleviates the need for culturing, and samples are analyzed in a more direct manner. This method is relatively easy, and many samples can be analyzed at the same time. Moreover, the results obtained by SDS-PAGE of whole-cell proteins can discriminate at the same level as DNA fingerprinting **[33]**.**[21]**used cluster analysis to compare protein banding patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 17 isolates of multinucleate *R. solani*(AG-4). A clear-cut relationship of protein profiles of the isolates with virulence, geographic origin, and source (cultivar used in isolation) was weak. Hence, the aim of this present investigation were to isolate some *A. solani* isolates which cause potato early blight disease from different Egyptian governorates and testing their pathogenicity on different potato varieties as well as, evaluate the *in vitro* and *in vivo* antagonistic activity of some bioagents against it. Finally, genetic variation among these isolates was determined using RAPD-PCR and SDS-PAGE techniques.

.

Paper elements:

MATERIALS AND METHODS

1. Isolation, purification, and identification of the causal pathogen

Pathogenicity test was carried out under greenhouse conditions using potato growing pots (50 cm diameter) (cv. Lady Rosetta). Samples of potato leaves, stems and roots showing typical symptoms of early blight disease were collected from five different Egyptian locations, i.e. Behaira (Koam Hamada), Gharbia (KaferElzayat), Manofiya (ShebenElkoam), Giza (Elbadrashen) and Minia (Elborgia) governorates. Isolation was carried out from the collected samples at the same day of collection as possible after storage in the refrigerator at 4-6°C for a few days. Infected tissues were cut into small pieces and were surface sterilized with sodium hypochlorite (0.5%) for 2-3 minutes, then, were washed for several times with sterilized distilled water. Small pieces from the edges of the sterilized pieces were dried between two sterilized filter papers and were transferred directly to the PDA medium in Petri dishes (9 cm) and incubated under 12h light and 12hdark at 25±1°C according to **[28]**.Pure cultures were maintained on PDA slants and were stored in a refrigerator at 5-10°C. The purified isolates were identified according to their morphological features using thedescriptions of **[40]**.

2. Preparation of soil, tuber, mycelial, spore suspension andPathogenicity test

2. a. Soil disinfestation

Sandy loam soil (50% sand and 50% loam w/w) wassterilized by using 5% formalin water solution and was covered with polyethylene sheets for 7 days, after which the cover will be removed. Sterilized soil was left for another 7 days for formalin evaporation.

2. b. Disinfection of tubers

Potato tubers surface was sterilized with sodium hypochlorite (1% active chlorine) for about 2 minutes and was washed several times by sterilized water, then was dried in between sterilized filter papers.

2. c. Mycelial fragments preparation

The inoculum of *A. solani* was prepared by culturing each of the obtained isolates on PDA medium at 25±2ºC for 10 days. Then 10 ml of sterile distilled water were added to each plate and colonies were carefully scraped with a sterile needle.The mycelial fragments were diluted using sterilized distilled water (1:2, w/v) and were used as an inoculum. The pots (50 cm diam.) were filled with the sterilized sand-loam soil (50% sand and 50% loam w/w) and were planted at the rate of 4 potato pieces/pot. The growing seedlings (45 days old) were sprayed using a hand atomizer according to the method of**[23]**.

2. d. Spores suspension preparation

Petri dishes (10 cm. diam.) have been prepared containing on 20 ml. V-8 juice agar medium and inoculate with *A. solani* isolates and incubated at 25±2°C for seven days of dark and at 30±2°C for 18h light, then at 15±2°C for 16 h. dark. The culture surface will be scratched after7 days of incubation. The conidia collected by scrapping the surface of growing cultures by using a sterilized needle. The spores will be then suspended into 15 ml. of sterilized water and filtered through a-cheesecloth to remove most of the mycelial fragments. The filtrate will be centrifuged at 3000 rpm for 3 min. This process will be repeated and the supernatant in each will be removed by a suitable amount of a-sterilized water. The concentrations of the spore suspension will be determined by aid a haemocytometer slide and adjusted to 5×106 spore/ml **[23]**.

2. e. Pathogenicity test

Pathogenicity test was performed under greenhouse conditions during the two growing seasons 2019/2020 and 2020/2021 using pots (50 cm. diameter), were filled with the sterilized sand-loam soil (50% sand and 50% loam w/w), planted at the rate of two pieces/pot of potato tubers having 3 eyes

of almost same size were arranged in the pot 5-7 cm below the soil surface (cv. Lady-Rosetta). Three pots were allocated to each isolate and pots containing sterile, uninoculated soil were used as control. Pots were arranged in a completelyrandomized design (CDR). All pots were kept under careful observations in greenhouse in natural light of Mallawy Metrological Station, Agricultural Research Center, Egypt. After two weeks of inoculation, disease severity was recorded following the scorechart 0-scale (0 = healthy; 1 = 1-5%; 2 = 6-10%; 3 = 11-25%; 5 = 25-50%, 7 = 51-75%, and 9 = >76% of the leaf area infected) according to **[25]**.

$$\text{Disease severity (\%)} = \frac{\Sigma (n \times r)}{NR} \times 100$$

Where:

n = Number of infected leaves on the plant.

N= Total number of leaves examined.

r = Numerical rate of infected leaves.

R= Highest numeric rate.

3. Molecular studies

The protein content in supernatant was estimated according in this experiment and carried out in the **(Molecular Biology laboratory of Assiut University, Assiut, Egypt)**. Isolates of *A. solani* (the causal organisms of early blight on potato) were grown in Petri dishes (4 cm) contained 1800 µL solid medium (potato dextrose agar) to which a layer of liquid medium 1400 µL of peptone yeast glucose was added. The fungal isolates were cultured by inoculating a small loop from stock onto the prepared Petri dishes that were subsequently incubated at 25±°C for 10 days. Mycelium was harvested from the medium using sterilized inoculating loops and was transferred into sterile 1.5 ml microcentrifuge tubes.

3.1. DNA isolation

The total genomic DNA was isolated using CTAB protocol for plants **[27], [38]**with some modifications. Each isolate was grown in 50 ml of potato broth (0.25 kg potatoes/1 L distilled water) for 5-7 days at room temperature in a rotary shaker set at 120 rpm. The mycelium of the five *Alternariasolani* isolates was collected by filtration. Cell walls of fungal mycelia were broken down by grinding with glass rods and in the presence of liquid nitrogen.

Ground fungal mycelia powder was transferred to 2 ml Eppendorf tubes. Then, 600 µl of 60ºC extraction buffer (100 mMTris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% hexadecyltrimethylammonium bromide (CTAB), adjusted to pH 8.0 and 50 µl β-mercaptoethanol were added to the samples, mixed by gentle inversion, and was incubated in 60ºC water-bath (with occasional gentle mixing) for 30 minutes. Samples were then removed from the water-bath and were cooled to room temperature for 4-6 minutes. An equal volume of chloroform: isoamylalcohol (24:1) was added to the cooled mixture then samples were mixed by gentle inversion to form an emulsion. Samples were centrifuged at 5000 rpm for 20 minutes at 10ºC. The supernatant was transferred to a new 1.5 ml Eppendorf tubes.

An equal volume of cold (-20ºC) isopropanol was added to the supernatant. Samples were then placed in a freezer (-20ºC) for one hour or left overnight at 4ºC to accentuate precipitation. Samples were centrifuged at 10,000 rpm for 5 minutes at 4ºC and the supernatant was poured, and the pellet was washed with cold 70% ethanol (v/v) and centrifuged thereafter at 10,000 rpm for 2 minutes at 4ºC. The latter step was repeated twice then the pellet was dried in an 37ºC incubator (under vacuum) or left overnight at room temperature. The pellet was

thereafter dissolved in 300-500 µl TE buffer (250 mMTris-HCl, 0.5M EDTA, (pH 8.4).

Dissolved pellets were then treated with 3 µl RNase A (10 mg/ml) and were incubated in 37ºC for 30 minutes (with occasional gentle mixing). Samples were then treated with 3µl proteinase-K (1mg/ml) and then were incubated at 37ºC for 30 minutes(with occasional gentle mixing). An amount of 300 µl chlorophorm**:**isoamyl alcohol (24:1, v/v) was added to 1.5 ml Eppendorf tube, mixed gently and then was centrifuged at 1000 rpm for 5 minutes at 20ºC. Then the supernatants were transferred to new Eppendorf tubes where two volumes of cold ethanol were added to the tubes and 1/10 volume of sodium acetate (3M) was added to the previous mixture, mixed and left for an hour in a freezer (-20º C). Samples were then centrifuged at 10,000 rpm for 10 minutes at 4ºC, drained and washed with 70% ethanol (v/v) as previously mentioned. Ethanol was then removed, and pellets were left to dry (under vacuum) or overnight then dissolved in 50-µl TE buffer. DNA dilutions were made to detect the optimum concentration for RAPD-PCR analysis.

RAPD analysis

Five commercial 10-mer primers were used for studding genomic DNA samples. The nucleotide sequences of these primers which have been used for RAPD-PCR analysis are shown in Table (1).

Table (1): Primer name, sequence, annealing temperature and number of cycles used for RAPD-PCR analysistemperature and number of cycles used for RAPD-PCR analysis

Primer Name	Sequence (5′-3′)	Annealing temperature (ºC)	Number of cycles
OPA-3	AGT CAG CCAC	36	35
OPA-8	GTG ACG TAGG	36	35
OPA-15	AGA TGC AGCC	36	35
OPC-18	TGC GCG ACTC	36	35
UBC-126	CTT TCG TGCT	36	35

DNA amplifications were performed in a final volume of 25 µl containing 1 µl of DNA template (25 ng/ µl), 2 µl of primer and 10 µl (10 pmol), 12.5 µl (10 pmol) of master mix *taq* DNA polymerase (Sigma Scientific Services Co., Egypt). The reaction volume was completed to 25 µl with sterilized deionized water. The reactions were preheated in one step of 5 min at 95°C followed by 35 cycles of 3 steps (DNA denaturation at 94° C for 1 min, primer annealing at 36° C for 1 min and primer extension at 72° C for 2 min, respectively) in each and a final subsequent cycle of post extension at 72°C for 5 min. The amplification products were resolved by gel electrophoresis on 2% agarose gels in Tris-acetate EDTA (TAE) buffer at 100 volts. Subsequently, gels were stained with Ethidium bromide (0.1g Ethidium bromide dissolved in 10 ml 1X TAE buffer) for 30 min., visualized on UV light and the photo-documentation was performed. DNA fragments' sizes were analyzed using the free software *GelAnalyzer 3* which is available free on the internet at *http://www.geocities.com/egygene***[17]**.

3.2. Biological analysis

SDS-PAGE analysis

The biochemical variations of the five *Alternariasolani*isolates was done using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The mycelial mats were crushed in pre-chilled sterilized pestle and mortar into fine powder with liquid nitrogen and transferred to eppindorf tubes, containing extraction buffer (Tris-HCl 0.1 M pH 6.8, glycerol 5%, sodium dodecyl sulphate (SDS) 0.5% and mercaptoehanol 0.1%). The tubes were allowed to stand for one hour on ice. The samples were then centrifuged at 10,000 rpm for 20 mins at 4ºC the clear supernatant was collected. Seven volumes cold acetone added and kept at-20°C for overnight to this supernatant . The

proteins were precipitated by centrifugation at 10000 rpm for 20 min at 4°C the pellet was washed two times with cold acetone and air dried. The pellet was dissolved in sample buffer for SDS-PAGE and samples were loaded after denaturation. The gel was stained in coomassie blue solution. Polyacrylamide gel was prepared from the stock solutions by mixing them in following proportion. Gel was poured immediately after addition of TEMED and APS for polymerization. 50 µl of crude protein sample was mixed with 2X sample buffer in 1:1 ratio. Sample was boiled in water bath for 5min. and centrifuged at 10,000 rpm for10min and loaded on gel. Molecular weight of marker in the range of 11-245 kDa (**4-15% TG SDS buffer**) was 10µl/well) used as standard. Gel was pre-run for 15min at 80V. Samples were loaded and electrophoresed at 80 volts till the sample is in stacking gel and voltage raised up to 120 volts for separating gel. Gel running was done till the dye reached 0.5cm (7-8 hrs.) from the lower edge of the gel. After the electrophoresis, the gel was stained in staining solution containing Coomassie brilliant blue (R 250) for overnight. The gel was de-stained in de-staining solution (3%Nacl).

4. Biological control of *Alternariasolani*

4. a. Antagonistic effect of some bioagents on the virulent isolate of *A. solaniin vitro*

Studying antagonistic effect of some microorganisms *i.e.,Trichodermaharzianum T. virid, Bacillus subtilis* and *Bacillus megaterium* on growth of the virulent isolate of *A. solani* in *vitro*. The isolates of fungi and bacteria that gave strong antagonistic effect were kindly brought from the Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.Trichoderma isolates were identified according to their morphological and microscopic characteristics **[12]**and confirmed by the Fungal Taxonomy Department, Plant Pathology Res. Inst., Agriculture Research Center, Giza, Egypt and were kept in slants of PDA for further studies. Whereas, the bacterial isolates were identified according to **[8]**.

A disc of *T. harzianum* and *T. viride*; isolate; a 0.5 cm in diameter was placed in PDA medium on both sides of Petri dishes. At the same time, the other side of each plate (on at the center) was inoculated with mycelia disc (5cm) at the age of three days of *A. solani*. Inoculated plates were incubated at 25±2°C, for five days. The obtained growth from streakof bacteria ; *B. subtilis, B. megateriumand* 3-day-old culture was placed at opposite sides of plate by using needle, at the same time, other side on at the center of each plate. Petri plates inoculated with either pathogen or antagonistic agent alone were served as control. Four replicates were used for each treatment. Inhibition zones were measured when the fungal growth was completely covered the plate surface (9.0cm in diameter) of the control treatment.

The radial growth inhibition percentage of the total percentage was calculated using Abbott equation **[15]**. according to this formula:

I.P= C **-** T ÷ C× 100

C= Radial growth of control,

T= Radial growth of treatment and

I.P= Inhibition%

4. b. Under greenhouse conditions

Evaluation of the effectiveness of these previously mentioned treatments on disease severity of potato plants (LadyRosetta) with early blight disease caused by *A. solani* under greenhouse and field conditions, was compared to chemical pesticides: Score 50% EC and Redomil plus (2g/L) before and after inoculation at the recommended concentrations under natural injury conditions in the field.

In this experiment, five antagonistic reagents were tested against the most aggressive isolate of *A. solani*. *T. harzianum* and *T. viride*were grown on PDA medium for 10 days, and then spores suspension of each fungus was prepared and adjusted to about 3×10^3 spores/ml with sterilized water using a haemocytometer slide. The tested bacteria; *B. subtilis* and *B. megaterium*were grown on nutrient medium for 3 days at 27°C. Susceptible potato plants (cv.LadyRosetta) were sprayed before and after inoculation with the microorganisms. Four replicates were used for each treatment. Percentages of the disease severity of *A. solani* were recorded after 15 days from spraying of spore suspensions of on potato leaves

4. c. Under field conditions

To test the efficiency of the bioagents to control potato early blight disease under field conditions, *T. harzianum* and *T. viride* were grown on PDA medium for 10 days and the tested bacteria; *B. subtilis* and *B. megaterium*were grown on nutrient medium for 3 days at 27°C. The susceptible potato plants (cv.LadyRosetta) were sprayed before and after inoculation with the tested bioagents. The average of disease severity of *A. solani* was recorded after 15 days from spraying of spore suspensions on potato leaves

5. Statistical analysis

Data were subjected to the statistical analysis of variance and the Complete Randomized Design (CRD) with three replications was used. The analysis of variance (ANOVA) of the obtained data was performed using the MSTAT-C program.

Gel images detected via PCR-based and SDS-PAGE were analyzed using the free software GelAnalyzer3 which is available free on the internet at *http://www.geocities.com/egygene***[17]**.Molecular size of the amplified fragments; presence (1) or absence (0) through samples, their frequencies through samples, and their polymorphism type either monomorphic or polymorphic as well as the mean of band frequency and the polymorphism percentage for each primer were determined. Data of the similarity matrix were used for cluster analysis using the software SPSS Ver. 11. Hierarchical cluster analysis was conducted using the PAST software version 1.88 **[19]**based on **[11]**.to determine the similarity coefficient matrix within the fungal isolates.

RESULTS

1. Isolation, purification, and identification of the causal pathogens.

One hundred plant samples show identical symptoms of tested diseases were collected randomly from each five different Egyptian locations, i.e., Minia, Behaira, Gharbia, Minofiya and Giza governorates as shown in Table (2). Natural symptoms (Figure 1) of early blight first appear as small irregular dark brown spots to circular on the lower (older) leaves turn yellow and die in potato (cv. Lady Rosetta). Therefore, cultivar Lady Rosetta; the highest susceptible potato cultivar was chosen for further studies.

Table (2): Isolates number and geographic origin of Alternariasolani isolates

No. isolate	Geographic origin
1	Minia (El-Borgia)
2	Behaira (Koam-Hamada)
3	Gharbia (Kafer El-zayat)
4	Minofiya (Sheben El-Koam)
5	Giza (El-Badrashen)

No. of fungal isolates	Disease severity %	
	Season 2019 /2020	Season202 0/ 2021
Control	0.00	0.00
1	56.19	44.30
2	32.38	25.71
3	22.86	21.90
4	16.19	9.52
5	8.57	4.76
L.S.D 0.05	6.56	3.74

A **B**

Figure (1): Natural symptoms of early blight potato (**cv. LadyRosetta**) plants. **A,** Potato leaves and **B,** tubers appear as small irregular dark brown spots to circular on the lower (older) leaves turn yellow.

2. Pathogenicity test

Data presented in Table (3) show that all the isolated tested isolates proved to be pathogenic to the tested potato (cv. Lady Rosetta); causing symptoms of early blight disease comparing with the control. In this regard, Minia (El-Borgia No.1) proved to be the highest aggressive isolate and was significantly the highest isolate causing early blight disease severity during 2019/2020 and 2020/2021growing seasons, respectively by about (44.30 and 56.19%), while isolates of Behaira (Koam-Hamada No.2), Gharbia (Kafer El-zayat No.3), and Minofiya (Sheben El-Koam No.4), governorates recorded the moderately disease severity by about (25.71 and 32.38%), (21.90 and 22.86%) and (9.52 and 16.19%), respectively compared to control under greenhouse conditions. On the other hand, isolate (El-badrashen No.5) recorded the lowest disease severity by about (4.76 and 8.57%).

Table (3): Pathogenicity test of A. solani isolates on susceptible potato plants (cv.Lady-Rosetta) under greenhouse conditions during 2019/2020 and 2020/2021 growing seasons.

No. of fungal isolates	Disease severity %	
	Season 2019/ 2020	Season 2020/ 2021
Control	0.00	0.00
1	56.19	44.30
2	32.38	25.71
3	22.86	21.90
4	16.19	9.52
5	8.57	4.76
L.S.D $_{0.05}$	**6.56**	**3.74**

3. Molecular results

DNA molecular genetic marker based on RAPD-PCR.

All RAPD primers produced scorable amplified bands in all the five Alternariasolani isolates except the UBC-126 primer which, did not produce any amplicons of isolate 5 of A. solani (Figure 3). A total of 22 amplified bands at sizes ranged from (125 to 2778 bp) were produced by ten RAPD primers with an overall mean of 4.8 ±1.02 and ranged from 2 bands of UBC-126 primer to 8 bands in OPA-3 primer (Table 4). From 22 generated bands only 16 bands were polymorphic with an overall mean of 3.2 ±0.97 and 8 bands were monomorphic with an overall mean of 1.6 ±0.51. The percentages of polymorphism among primers ranged from 50 to 100% with an overall mean of 67.5±10.90 as shown in Table 4.

Table (4): Primers, fragment size, monomorphic bands, unique bands, total number of polymorphic and bands and polymorphism % obtained by using ten RAPD primers

Only the RAPD amplicons produced by primers **UBC-126** exhibited 100% polymorphism of all tested fungal isolates, while the three primers (**OPA-8,OPA-15 and OPC-18)** showed 50%

Primers	Fragment size (bp)	Monomorphic bands	Unique bands	Polymorphic bands		Total number of bands	Polymorphism (%)
				without Unique	with Unique		
OPA-3	125-2626	1	0	7	7	8	87.5
OPA-8	324-2672	2	0	2	2	4	50
OPA-15	433-2320	3	0	3	3	6	50
OPC-18	664-2778	2	0	2	2	4	50
UBC-126	1469-2512	0	0	2	2	2	100
Total		8	0	16	16	22	
Mean ± SE		1.6 ± 0.51	0	3.2 ± 0.97	3.2 ± 0.97	4.8 ±1.02	67.5± 10.90

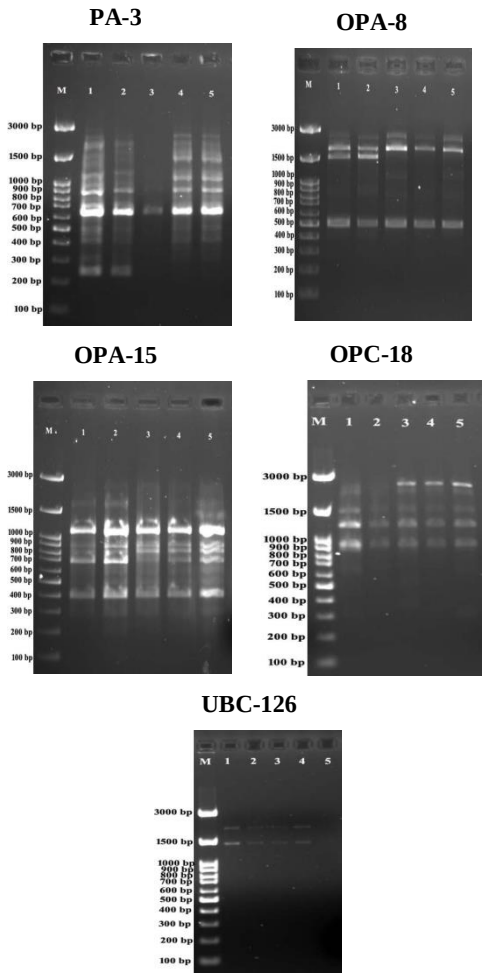

Figure (3): Electrophoretic gel patterns of RAPD DNA products generated by OPA-3, OPA-8, OPA-15, OPC-18 and UBC-126 primers. Lane M, DNA Ladder 100 bp; Lane 1, fungal isolate 1; lane 2, fungal isolate 2; lane 3, fungal isolate 3; lane 4, fungal isolate 4 and lane 5, fungal isolate 5.

polymorphism. Data in **Table 5** revealed that, all the RAPD primers did not produce any unique bands.

The number of fragment bands detected by any of the used RAPD primers depended on primer sequence and the extent of variation of the examined genotype(s). From the above-**mentioned** results, it can be concluded that the 5 utilized primers generate relatively polymorphism within the five studied *A. solani* isolates. fungal isolate 2; lane 3, fungal isolate 3; lane 4, fungal isolate 4 and lane 5, fungal isolate 5. Figure (3): Electrophoretic gel patterns of RAPD DNA products generated by OPA-3, OPA-8, OPA-15, OPC-18 and UBC-126 primers. Lane M, DNA Ladder 100 bp; Lane 1, fungal isolate 1; lane 2, fungal isolate 2; lane 3, fungal isolate 3; lane 4, fungal isolate 4 and lane 5, fungal isolate 5.

The resulted data from RAPD analysis were used in the estimation of genetic relationships among five different isolates of A. solani through a UPGMA cluster analysis of genetic similarity matrixes. Cluster analysis was achieved based on Dice's similarity coefficient matrix. The results revealed that the highest similarity value (0.923) was found between isolate 4 and isolate 5. On the contrast, isolate 2 and

isolate 3 exhibited the lowest value (0.667), as shown in Table 5.

Table (5): Dice's similarity coefficient matrix (1945) within the five isolates of *A. solani* based on bands polymorphism of RAPD primers.

Isolates	1	2	3	4	5
1	-	-	-	-	-
2	0.900	-	-	-	-
3	0.686	0.667	-	-	-
4	0.829	0.821	0.765	-	-
5	0.800	0.737	0.727	0.923	-

Data in Table 5 revealed that, all the RAPD primers did not produce any unique bands.

The number of fragment bands detected by any of the used RAPD primers depended on primer sequence and the extent of variation of the examined genotype(s). From the above-mentioned results, it can be concluded that the 5 utilized primers generate relatively polymorphism within the five studied A. solani isolates.

In order to determine the genetic variability among the five Alternariasolani isolates, matrix of **[10]**.similarity coefficient (S) was calculated based upon band sharing frequency (BS) as shown in Table 5. The dendrogram was constructed using the hierarchical cluster analysis method with the average linkage between pairs from the matrix of **[11]**. and similarity coefficient values (S) within the five fungal isolates (Figure 4).

All the five tested fungal isolates were distributed by the dendrogram into two main clusters; the first one was related to isolate 3; while, the second cluster subdivided into two main groups; the first one splitted to two sub-clusters; the first one was for isolate 1 and the other one was for isolate 2. Moreover, the second group was splitted in two sub-clusters; the first one was for isolate 4 however, the second sub-cluster was related to isolate 5.

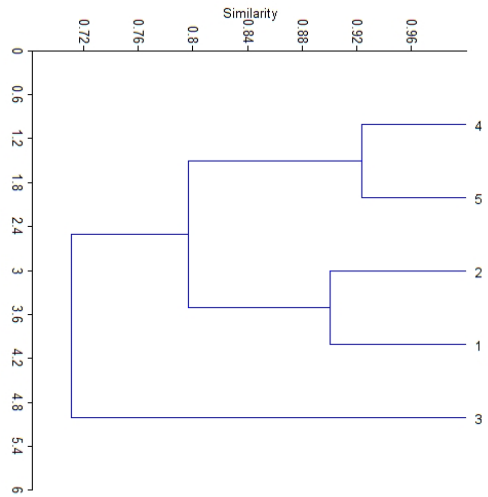

Figure (4): The dendrogram of genetic relationships among of the five isolates A. solani based on RAPD markers

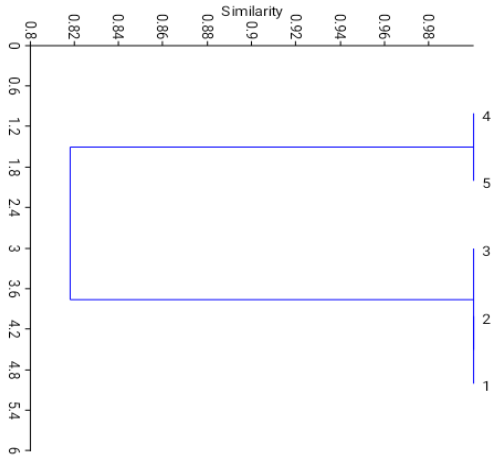

Figure (6): The dendrogram of genetic relationships among the five isolates of *A. solani* based on SDS-PAGE protein patterns.

Biochemical analysis

Soluble proteins banding patterns of five A. solani isolates have shown in Figure 5. A total of 13 bands were observed with molecular weight between

19.46-126.48 KDa. All the proteins are not present in equal abundance. The highest bands (13) were found in the fungal isolates 3, 4 and 5 which exhibited no variation in proteins, while, the lowest bands (9) were found in the two (1 and 2) isolates which did not exhibit more proteins as compared to other isolates. Dendrogram was constructed using the hierarchical cluster analysis method with the average linkage between pairs from the matrix of **[11]**.and similarity coefficient values (S) within the five fungal isolates (Figure 5).

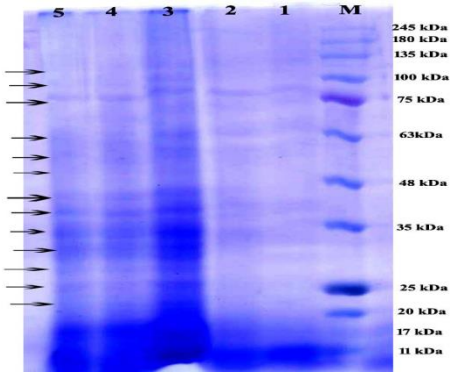

Figure (5): SDS-PAGE protein patterns of five *A. solani* isolates. **Lane M,** Standard Marker proteins; **Lanes** (1 to 5) fungal isolates.

All the five tested fungal isolates were distributed by the dendrogram into two main clusters; the first one was subdivided into three groups the first one was for isolate 1 and the second was for isolate 2 finally the third, was for isolate 3. On the other side, the second cluster was splitted to two groups the first one was for isolate 4 however, the second was related to isolate 5.

3. Antagonistic effect of some bio-agents on the virulent isolate of A. solani in vitro

Data presented in Table (6) and illustrated in Figures (7 and 8) show that T. harzianum was the best antagonist against A. solani growth compared with other treatment and control. It significantly showed the highest inhibition zone (67.33cm) with the highest aggressive isolates of A. solani; followed by T. viride (64.22cm), while B. subtilis and B. megaterium proved the second grade (44.52 cm). On the other hand, B. megaterium gave the lowest inhibition zone (26.59 cm). Data show that there were no noticed significant differences among the tested bio-agents.

Table (6): Antagonistic effect of some bio-agents on the virulent isolate of A. solani in vitro.

Treatment	Inhibition zone (cm.)
Control	0.00
T. harzianum	67.33
T. virid	64.22
B.subtilis	44.52
B. megaterium	26.59
L.S.D $_{0.05}$	**10.09**

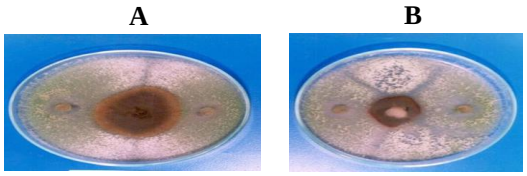

Figure (7): Antagonistic effect of Trichodermaharzianum against the highest (A and B) aggressive isolates of A. solani.

Figure (8): Antagonistic effect of *Bacillus subtilis* against the highest (A and B) aggressive isolates of *A. solani*.

4. Evaluation of the efficacy of some bio-agents against infection with A. solani of potato plants (cv. Lady Rosetta)

4. a. Under greenhouse conditions

Data presented in the Table (7) elucidate that all the tested microorganisms have an efficient effect in

controlling this disease; they equally decreased the percentage of disease severity in contrast to the check plants and showed the reduced percentage of potato early bight disease. In this respect, the same data indicated that the highest reduction of the disease severity was proved by T. harzianum by about (19.77 and 11.25%) followed by T. viride, (35.58 and 24.13%), respectively, during the seasons of 2020/2021 and 2021/2022, respectively compared with B. subtilis and B. megaterium by about (42.87, 29.54 and 43.56, 41.61 %), and control by (62.29 and 60.00%), respectively.

Table (7): Effect of some bio-agents against infection with A. solani of potato plants (cv. Lady Rosetta) under greenhouse conditions during 2020/2021and 2021/2022 growing seasons .

Treatment	Disease severity %	
	Season 2020/2021	Season 2021/2022
Control	62.29	60.00
T. harzianum	19.77	11.25
T. virid	35.58	24.13
B. subtilis	42.87	29.54
B. megaterium	43.56	41.61
L.S.D $_{0.05}$	**9.10**	**3.86**

4. b. Under field conditions

Data presented in Table (8) showing the effect of spraying potato plants, cv. Lady Rosetta with some antagonists on naturally early blight indicated that the treatments which were added during 2021/2022 growing season gave the best results in reduction of the disease in comparison with that of the 2020/2021 growing season. In that regard, T. harzianum gave the best results by about (25.32 and 10.66%) followed by T. viride by about (36.63 and 21.14%) compared with B. subtilis and B. megaterium by about (39.84, 27.66 and 40.38, 36.51) and control by about (60.57 and 59.98%), respectively.

Table (8): Effect of some bioagents against natural infection with A. solani of potato plants (cv. Lady Rosetta) under field conditions during 2020/2021 and 2021/2022 growing seasons.

Treatment	Disease severity %	
	Season 2020/2021	Season 2021/2022
Control	60.57	59.98
T. harzianum	25.32	10.66
T. virid	36.63	21.14
B.subtilis	39.84	27.66
B. megaterium	40.38	36.51
L.S.D $_{0.05}$	**6.77**	**4.73**

DISCUSSION

Five isolates of A. solani were isolated from potato varieties, Spunta and Lady Rosetta from different localities as above mentioned. These results are in consistency with those obtained by Kuczynska (1983) who reported that the most pathogenic fungi isolated from leaves and tubers of potato were A. solani and A. alternata.

Likewise, the pathogenicity test indicated that all the tested A. solani isolates were pathogenic to potato plants causing typical symptoms of the early blight disease. Moreover, data showed that Minia (El-Borgia No.1) proved to be the highest aggressive isolates of the disease severity while isolates of Behaira (Koam-Hamada No.2), Gharbia (Kafer El-zayat No.3), and Minofiya (Sheben El-KoamNo.4), governorates recorded the moderately disease severity. Meanwhile, El-badrashenNo.5 recorded the lowest disease severity. Similar results about the isolates of A. solani and variations among them were obtained by **[9]**.

The level of genetic diversity among fungal isolates could be attributed to mutations, recombinations, and/or the widespread movement of this pathogen over large distances. It has been hypothesized that asexual recombination through the parasexual cycle may be the source of genetic

variation in A. solani, although currently it is unknown if this occurs commonly within Alternaria species **[32]**and **[44]**. The present data are in agreement with those reported by **[3]**, **[45]**and **[18]**. The results obtained with electrophoresis supported the use of these macromolecular criteria as an aid in differentiating the differences of Alternaria isolates encountered on various vegetable, spice and oil yielding crops. Electrophoretic patterns of soluble proteins were shown to be more valuable as diagnostic aids in distinguishing fungal isolates whose variability in morphology is indeterminate and overlapping **[34]**, **[31]**and **[5]**. Thus, polyacrylamide gel electrophoresis of soluble mycelial protein patterns obtained in this study can be used as a reproducible and sensitive fingerprints for the five Alternaria isolates infected potato plants.

Also, **[41]**found that one primer; P248, generated DNA polymorphisms that distinguished A. solani and A. alternate with an approximate 97% confidence. Also, they found that a significantly large genetic distance between isolates of A. solani from potato and tomato, perhaps indicative of pathogenic specialization in this species. Similar trends of genetic variation among isolates were also reported by many investigators **[43]**and **[41]**. It was observed that a wide genetic variation among the five A. solani isolates were evident due to the high number of polymorphic bands. In case of RAPD-PCR, the primer UBC-126 gave a respectively polymorphic xwhile it was decreased into 50% with the primers OPA-08, OPA-15 and OPC-18. Furthermore, data obtained by RAPD-PCR grouping the A. solani isolates were divided into two large clusters. The electrophoresis profiles of the five isolates of A. solani showed differences in the protein molecules. Grouping the isolates based on PAGE analysis was not associated with the geographic origin of isolates because the two isolates of Minia (El-Borgia No.1), and Behaira (Koam-Hamada No.2) showed the highest similarity, while the isolates of Giza (El-badrashen No.5) showed lowest one.

Like that, Protein patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for the 5 isolates of A. solani were divided into sub-clusters; the tested isolates were separated into groups based on their protein profiles; however, grouping the isolates was not related to their pathogenicity and geographic origins. Thus, the isolate of Minia (El-Borgia No.1) was the high level in protein formation, but, this may be due to trade and movement of potato and the pathogen may be introduced by the activity of human or this is may be due to geographic origin of the isolate. **[4]**who pointed out this lack of relationship among proteins electrophoretic. Thus, the isolate; Minia (El-Borgia No.1) showed the highest performance. These results agree with those of **[30]**who found that the spore and mycelial allergens of the two species of Alternariabrassicicola and A. alternate extracted under two different conditions were analyzed by radio rocket immunelectrophoresis and crossed radioimmuoelectrophoresis. Furthermore, RAPD-PCR, SDS-PAGE protein patterns and phylogenetic analysis showed that isolates 1, 2 and 3 that have the highest pathogenicity were similar to each other and shared the same cluster while the other fungal isolates 4 and 5 with low pathogenicity were close to each other. So, data approved that there were no relationship between pathogenicity and the geographic distribution of all tested A. solani isolates.

Finally, mmore attention was focused on Biological control of A. solani by T. harzianum, T. viride, B. subtilis and B. megaterium. These microorganisms showed strong antagonistic effects against the pathogenic fungus A. solani in vitro and reduced the disease severity of early blight disease.

The above-mentioned results are generally in agreement according to **[6]**who found that T. harzianum and T. viride were significant effective in inhibiting the mycelial growth of A. solani. There is no significant difference between the effectiveness of Trichoderma species in pot culture studies. Like that, **[7]**tested six strains of P. fluorescens in vivo and in vitro for their biological control potentially against A. solani, and all strains of P. fluorescens inhibited A. solani growth (by 28-40%) compared with the control.

On the other hand, **[1]**found that B. subtilis was the most effective antagonist in reducing A. solanimycelial growth, followed by T. harzianum in vitro against A. solani growth isolated from early blight infected potato leaves. Similar results were obtained by **[13]**; who stated that spraying potato plants with B. subtilis strain Nj-18 showed that it could colonize the plants. They inoculated the plants with the spore suspensions of A. solani after spraying the fermentation of Bacillus NJ-18. The results were recorded after 14 days and the efficacy in controlling early blight disease was 72.9%. The above-mentioned results are in general agreement according to **[23]**who mentioned that T. harzianum was the best antagonist against A. solani growth, followed by T. Koningii, T. hamatum and T. Longibrachiatum, while B. subtilis registered the second grade and proved application of the bioagents individually proved the highest inhibition zone in vitro and reduced disease severity of early blight disease of potato under greenhouse and field.

In vitro, the most effective evaluation method of A. solani is the use of microorganism i.e.,Trichoderma, viride, Bacillus subtilis and Bacilus. megaterium. As well as, application of bioagents against early blight in potato plant (cv. Lady Rosetta) infected with A. solani under greenhouse and field conditions. Recently an ecofriendly biocontrol agent, Bacillus subtilis has received much attention by both conventional and organic farmers to suppress plant diseases **[48]**; **[37]**. Biological control of A. solani with Trichoderma sp. has been proved more effective and environment friendly **[16]**.Trichoderma species besides inhibiting growth of fungi also promote growth and development of plant **[39]**. T. harzianum and T. viride inhibiting the mycelial growth of A. solani**[6]**. **[7]**tested six strains of Pseudomonas fluorescns in vivo and in vitro for their biological control potential against A. solani. Evaluated B. subtilis and Trichodemaharzianum in vitro against A. solani growth isolated from early blight infected potato leaves **[1]**. Kuczynska (1983) mentioned that the most pathogenic fungi for leaves and tubers of potato were A. solani and A. alternate. A. solani isolated from potato leaves was pathogenic independently to 3 potato cultivars in 1983 and 1984 **[22]**. **[29]**found variability among 109 isolates of A. solani in their pathogenicity to potato plants. Their fungal isolates were classified according to the necrotic leaf area as lower, middle and highly aggressiveness.

Conclusions

In conclusion, the results obtained of pathogenicity showed that all of the tested isolates proved to be pathogenic to the tested potato (cv. Lady Rosetta) causing symptoms of early blight comparing with the control. The fungal isolate No. 1 which was obtained from (El-Borgia, Minia governorate) was the most aggressive isolate with the highest significant values of disease severity (44.30 and 56.19%) during the two successive seasons of (2019/2020) and (2020/2021), respectively.

The results obtained by RAPD-PCR, SDS-PAGE protein patterns and phylogenetic analysis showed that isolates 1, 2 and 3 that have the highest pathogenicity were similar to each other and share the same cluster, while the other fungal isolates 4 and 5 with low pathogenicity were close to each other. Also, data showed that there is no relationship between pathogenicity and the geographic distribution of all tested A. solani isolates.

The antagonism of some living organisms such as: (Trichodermaharizianum, Trichodermavirde) and (Bacillus subtilis and B. megaterium)were studied in vitro against mycelial growth and disease severity of potato early blight under greenhouse and field conditions were studied in two successive seasons. Results indicated that the in vitro antagonistic testshowed that Trichodermaharzianum was the best bioagent that gave the highest inhibition zone (67.33cm) against the most aggressive A. solani isolates as compared with all other bioagents and gave the lowest values of early blight disease severity during the two seasons (19.77, 11.25) and (25.32 and 10.66%, respectively)compared to the other treatments, the control (infected and untreated potato plants).

REFERENCES

1.Abdul Hafeez; M. A. Khan and R.A. Chohan. , 2001.*In vitro* evaluation of various antagonists and plant extracts against mycelial growth of *Alternariasolani*. Pakistan Journal of Phytopathology, 13(2):127-129.

2.Abuley, I. K. and Nielsen, B. J. , 2017.Evaluation of models to control potato early blight (*Alternariasolani*) in Denmark. Crop Protection, 102, 118-128. https://doi.org/10.1016/j.cropro.2017.08.012

3.Adss, I. A; Abdel-Gayed, M. A.; Botros, W. and Hafez, E. E., 2017.Multilocus Genetic Techniques, RAPD and ISSR Markers, and Polygalacturonase Activity as Tools for Differentiation among *Alternariasolani* Isolates on Tomato Fruits and Relation to Their Pathogenicity in Egypt. Asian Journal of Plant Pathology, 11(1):18-27.

4.Aly, A. A.; S. H. Mostafa and M. T. M. Mansour., 2001.Effect of powdery mildew disease on yield components of some flax lines. J. Agriculture Science Mansoura Univ., 26:7711-7725.

5.Andrew, M.; Peever, T. L. and Pryor, B. M., 2009.An expanded multilocus phylogeny does not resolve species within the small-sporedAlternaria species complex. Mycologia. 101:95-109.

6.Babu, S.; K. Seetharaman; R. N. Kumar and L., Johnson., 2000b.Efficacy of fungal antagonists against leaf blight of tomato caused by *A. solani.* Journal of Biological Control, 14(2):79-81.

7.Babu, S.; K. Seetharaman; R. N. Kumar and L., Johnson., 2000c.Biocontrol efficacy of *Pseudomonas fluorescens* against *A. solani* and tomato leaf blight disease. Annals of Plant Protection Sci., 8(2):252-254.

8.Burbage, DA; Sasser, M and Lumsden, RD., 1982.A medium selective for Pseudomonas cepacia (Abstrac.). Phytopathology, 72: 706.

9.Burm, L. H.; Yu-seun Hun, H. B. and Yu, S. H., 1995.Incidence of Alternaria species in red pepper and sesame from Korea and their ability to produce mycotoxins. Korean J. of Plant Pathol, 11 (1):1-8.

10.Christ, BJ and Haynes, KG., 2001.Inheritance to early blight disease in a diploid potato population. Plant Breed 120: 169-172.

11.Dice, L. R., 1945.Measures of the amount of ecologic association between species. Ecology, 26: 297-302.

12.Domsch, KH; Games, W and Traute-eidi, A., 1980.Compendium of soil fungi. Academic Press A subsidiary of Harcout Brace Jovanovich, Publisher, London, 1: 859pp.

13.Dong, Y.; W. Jian; X. Z. Ming and M. Guo., 2009.Biological control of *A. solani* by *Bacillus subtilis* Nj-18. College of plant protection, Nanjing Agricultural University, Nanjing, Jiangsu, 210095, China.

14.Food and Agriculture Organization Corporate Statistical Database., 2016.Rome, Italy.

15.Frolich, G., 1979.Phytopathology und pflanzenschutz. VEB. Gustav Fisher Veriag. Jena, 295pp.

16.Gardener, M.B.B. and D.R. Fravel., 2002. Biological control of plant pathogens: Research, commercialization, and application in the USA. Online. Plant Health Progress doi: 10.1094/PHP-2002-0510- 01-RV

17.GelAnalyzer, version three., 2007. Gel analyzer vrs.3 program software for windows. www. Geocities .com/ egygene.

18.Guo, L.D.; L. Xu; W.H., Zheng and K.D., Hyde., 2004.Genetic variation of *Alternaria alternate*, an endophytic fungus isolated from Pinustabulaeformis as determined by random amplified microsatellites (RAMS). Fungal Diversity, 16: 53-65.

19.Hammer, Ø. A. T. Harper and P.D. Ryan., 2009.PAST-PAlaeontologicalSTatistics, ver. 1.88. http://folk.uio.no/ohammer/past

20.Hill, J. and Lazarovits, G., 2005.A mail survey of growers to estimate potato common scab prevalence and economic loss in Canada. Can. J. Plant Pathol. 27: 46-52.

21.Hussein, E.M., A.D.A. Allam, A.A. Aly, A.M. Amein, and A.M.A. El-Samawaty., 2000. Separation by protein electrophoresis of *Rhizoctonia* spp. isolated from cotton seedlings. J. Agric. Sci., Mansoura Univ. 25: 4035-4046.

22.Johnson, K.B.; P.S. Teng and S. Radcliffe., 1987.Analysis of potato foliage losses caused by interacting infestations of early blight, Verticillium wilt and potato leafhopper and the relationship to yield. Zeitschrift fur Pflanzenkrankheiten and Pflanzenschutz, 94: 22-23 (c. f. CAB Abstracts 1987-1989).

23.Khalil, M.A., 2012.Pathological studies on some fungal diseases of potatoes with special reference to their control in Egypt. Ph.D. Thesis, Department of Plant Pathology, Faculty of Agriculture, Al-Azhar, University, p.p.1-177.

24.Kumar, V.; Haldar, S.; Pandey, K.K.; Singh, R.P.; Singh, A.K. and Singh, P.C., 2008.Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of *Alternariasolani* in India. World Journal of Microbiology & Biotechnology 24(7): 1003-1009.

25.Latha, P.; Anand, T.; Ragupathi, N.; Prakasam, V. and Samiyappan, R., 2009.Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternariasolani*. Biological Control. 50(2): 85-93.

26.Morris, P. F.; Connolly, M. S. and St Clair, D. A., 2000 .Genetic diversity of

Alternariaalternata isolated from tomato in California assessed using RAPDs. Mycological Research, 104, 286-292.

27.Murray, M. G. and Thompson, W. F., 1980 .Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, 8 (1), 4321-4325.

Mycological Research, 104:151-160.

28.Naik, M.K.; Prasad, Y.; Bhat, K.V. and Rani, G.S., 2010.Morphological, physiological, pathogenic and molecular variability among isolates of *A. solani* from tomato. Indian Phytopathology, 63(2): 168-173.

29.Perez, S. 2003.Cultural, pathogenic and genetic variability of the causal pathogen of early blight (*Alternariasolani*Sor.) on tomato (*Lycopersiconesculentum* Mill.) in Cuba. Revista de Protection Vegetal, 18(3): 216.

30.Paris, S.; C. Fitting; B. S. E. Ramirez; J.P. Latge and M.D., David., 1990.Comparison of different extraction methods of Alternaria allergens. Immunol. 85(5):941-948.

31.Peever T. L.; Su G. and Carpenter-Boggs L., 2004.Molecular systematics of citrus-associated *Alternaria* species. Mycologia, 96(1):119-134.

32.Petrunak, D. M. and Christ, B. J., 1992.Isozyme variability in *Alternariasolani* and *A. alternata*. Phytopathology, 82, 1343-1347.

33.Priest, F.G. and Austin, B., 1993.Modern Bacterial Taxonomy (2nd ed.) Chapman & Hall, London.

34.Pryor, B. M. and Bigelow, D. M., 2003.Molecular characterization of Embellisia and Nimbyaspecies and their relationship to Alternaria, Ulocladium, and Stemphylium. Mycologia 2003; 95:1141-1154.

35.Roberts, R.G.; Reymond, S.T. and Anderson, B., 2000.RAPD fragment pattern analysis and morphological segregation of small spored*Alternaria* species and species group.

37.Romero, D.; A. deVicente; R.H., Rakotoaly; S.E., Dufour; J.W., Veening; E., Arrebola; F.M., Cazorla; O.P., Kuipers; M., Paquot and A., PérezGarcía., 2007. The Iturin and Fengycin Families of Lipopeptides are key factors in Antagonism of *Bacillus subtilis* toward Podosphaerafusca. Amer. Phytopathol. Soc., 20: 430-440.

38.Saghai-Maroof, M. A.; Soliman, K. M.; Jorgensen, R. A. and Allard, R. W., 1984.Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc.Natl. Acad. Sci. USA 81: 8014-8018.

39.Samuels, G. J., 2006. *Trichoderma*: systematics, the sexual state, and ecology. Phytopathology, 96: 195-206.

40.Singh, R.S., 1982.Plant Pathogens "The Fungi" Oxford and IBH publishing Co. New Delhi, Bombay Calcuta, 443pp.

41.Tiffany, l. Weir; David, R. Huff and Barbara, J. Christ; C. Peter Romaine.,1998.RAPD-PCR analysis of genetic variation among isolates of *Alternariasolani* and*Alternariaalternata* from potato and tomato. Mycologia, 99 (5): 813-821.

42.Tiwari, G. P.; Tiwari, SP; Nema, S. and Tripathi, N., 2017.Identification of Molecular and Biochemical Variations in *Alternaria* spp. International Journal of Chemical Studies, 5(5): 735-739.

43.Vakalounkis, D. J., 1991. Control of early blight of greenhouse tomato caused by *Alternariasolani* by inhibiting sporulation wih ultraviolet-absorbing vinyl film. Plant Dis., 75 (8): 795-797.

44.van der Waals, J. E.; Korsten, L., and Slippers, B., 2004.Genetic diversity among *Alternariasolani* isolates from potatoes in South Africa. Plant Disease, 88, 959-964.

45.Weber, B. and Halterman, D. A., 2012.Analysis of genetic and pathogenic variation of *Alternariasolani* from a potato production region. Eur. J Plant Pathol, 134:847-858.

46.Weir T. L.; Huff D. R.; Christ, B. J. and Romaine, C. P., 1998. RAPD-PCR analysis of genetic variation among isolates of *Alternariasolani* and *Alternariaalternata* from potato and tomato. Mycologia, 90:813-821.

47.Wilson, C. R., 2004.A summary of common scab disease of potato research from Australia. In: Naito S, Kondo N, Akino S, Ogoshi A, Tanaka F, eds. Proceedings of the International Potato Scab Symposium 2004. Sapporo, Japan: Hokkaido University, 198-214.

48.Zitter, T.A.; J.L. Drennan; M.A., Mutschler and M.J., Kim., 2005. Control of Early Blight of Tomato with Genetic Resistance and Conventional and Biological Sprays. Acta Hort. (ISHS), 695: 181-190.