Tikrit Journal for Agricultural Sciences (2021) 21 (2):112-129
https://doi.org/10.25130/tjas.21.2.12

IRAQI Academic Scientific Journals
العراقية المجلات الأكاديمية العلمية

ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

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KEY WORDS:

Strawberry fruits, fungi, fungal frequency, plant extracts and chemicals.

ARTICLE HISTORY:

Received: 12 /04/2021

Accepted: 20 /05/2021

Available online: 30/06/2021

Identification of Fungi Associated with Strawberry Fruits Taken from Sulaimani Markets and their In Vitro Management Techniques.

ABSTRACT

Fruit samples of strawberry were collected from local markets in Sulaimani Governorate/Iraq which they are imported from Turkey and Iran.Two different methods for identifying the fungi were used; morphologically, depending on some characteristic such as the shape of the spores, the color and the general shape of the fungus. Also to confirm the diagnosis fungal, molecular method was used. The mycotic observations showed that there were nine distinct fungi. The molecular data was blasted at NCBI checked and the identification rate was between 97-100%. The diagnosed fungi were (Alternaria alternate, Aspergillus niger, Botrytis cinerea, Cladosporium cladosporioides, Fusarium oxysporum f. sp. fragariae, Nigrospora sphaerica, Penicillium raistrickii, Penicillium griseofulvum and Rhizopus stolonifer). The highest infestation percent was recorded for A. niger 26.02%, while the lowest was for Fusarium oxysporum f. sp. fragariae 1.08%, pathogenicity test was performed for the isolated fungi and revealed that all of the fungi were pathogenic more than 90%. Samples from local markets showed no significant differences in disease incidence and the severity of the disease. For management of the fungi associated with strawberries, different method have been used in vitro (rosemary and pomegranate) plant extracts, salicylic acid (SA), potassium metabisulphite (KMS) and two fungicides. 1% and 2% rosemary plant extracts gave the highest inhibition of (P. griseofulvum) also, pomegranate peel extract 4% had a significant effect on (N. sphaerica). In addition SA were effective on all fungi and KMS had the same as the fungi excepted (R. stolonifera) compared to control treatment.

INTRODUCTION

Strawberry (Fragaria ananassa Duch.) (Rosaceae) grows in the most arable areas of both the Southern and Northern Hemisphere (Stauct, 1989 and Potter et al., 2007)

Fungal disease after harvest is one of the main factors limiting the marketing and shelf life of strawberry fruits, which also results in severe economic losses worldwide, as well as having a short postharvest shelf-life and market life, susceptible to mechanical injury, high respiration levels, sensitivity to fungal spoilage, physiological disorders, and decay (Amal et al., 2010, Patil and Suryawanshi, 2014, and Feliziani and Romanazzi, 2016).

The strawberry in the field is affected by a large number of diseases caused by fungi, bacteria, viruses, and nematodes. These pathogens cause damage to all part of plants and fruits, therefore caused heavy economic losses (Maas, 2004).

According to the American Phytopathological Society (APS), Common type of injuries related to fungal pathogens.These are several species of fungi capable of causing damage to more than one

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part of the strawberry plant (leaf, root, crown, and/or fruit) (Husaini and Neri, 2016). The main strawberry pathogen is Botrytis cinerea, followed by Rhizopus stolonifer, Mucor spp., Colletotrichum spp., and Penicillium spp. (Feliziani and Romanazzi, 2016).

In general, control of fungal phytopathogens is performed by applying synthetic fungicides. However, their use is harmful to the environment, human and animal health (Nunes, 2012).
Recently, the use of fungicides is less acceptable by the consumer, therefore, the recent trend is shifting toward safer and more development, environmentally friendly alternatives for the control of postharvest decay (Morsy et al., 1999). Therefore, several studies have been focused on using environmental friendly methods to control the fungal diseases to reduce synthetic fungicides. Some of these techniques use plant extracts from the different parts of certain plant species have been successfully tested to demonstrate their anti-fungal activities (Bowers and Locke, 2000).

Nowadays, postharvest life of strawberries can be extended with natural substances for instance; rosemary extract (Rosmarinus officinalis L.). Thus, it is edible, inexpensive, environment-friendly, antioxidant, antimicrobial anti-inflammatory and health benefits. It can be caused to prolong shelf life of strawberry through a reduction of moisture loss; gas exchange; respiration rate and oxidative reaction rates (Erkan et al., 2008, Genena et al., 2008, and Pour et al., 2014). As well as, more pomegranate peel medicinal qualities such as antibacterial, anti-fungal, antiviral, and antioxidant, have been investigated in recent years (Heber et al., 2006), also, other natural products have been evaluated by Dahham et al. (2010) as sources of antibacterial and anti-fungal activities of pomegranate peel extract (rind), seed extract, juice and whole fruit on the selected bacteria and fungi.

On the other hand, many of these salts are effective against a wide range of microorganisms; they have potential to control postharvest disease, salt treatments may inhibit plant pathogens or suppress mycotoxin production (Olivier et al., 1999), potassium metabisulfite ($K_2S_2O_5$) is commonly used in households for wine making and also for preserving all kinds of fruits and vegetables (Sethi, 2007), and Salicylic acid (SA) represents an interesting new opportunity in controlling fungal diseases within an environmental friendly integrated crop protection system by improving the plant's resistance to pathogen and inhibiting the growth of various pathogen (Ellis et al., 2002).

The objectives of the present study were to investigate, isolate and identify the fungi by morphological and molecular (Polymerase chain reaction PCR) associated with strawberry fruits taken from local markets (imported from neighboring countries), and using eco-friendly tactics to control the isolated fungi by in vitro using plant extracts and, chemicals.

MATERIAL AND METHODS

SAMPLING OF STRAWBERRY FRUITS AT VARIOUS LOCAL MARKETS IN SULAIMANI

Thirty–one strawberry fruit samples were randomly collected from local markets in Sulaimani Governorate from (April to July 2019) by taking the average samples for each month with four replicates. Samples were stored separately in clean plastic bags and they were written some information such as (date and area name), transferred to the College of Agricultural Engineering Sciences at the University of Sulaimani and stored in the refrigerator (4oC) for one day until analysis process. The samples sources were imported from (Turkey and Iran).

ISOLATION, PURIFICATION AND IDENTIFICATION OF THE FUNGI ASSOCIATED WITH STRAWBERRY FRUITS

Isolation and purification

Strawberry fruits pathogen (s) was isolated from infected strawberries, a small portion (1 cm2) of the fruits were cut with a sterilized scalpel, superficially sterilized in sodium hypochlorite (2%NaOCl) solution for two min., then washed three times with sterilized distil water to get rid of NaOCl residues, and dried with 9 mm sterilized filter paper, then the specimens were plated on PDA-medium and incubated at (25± 2oC) for 7 days (Chliyeh et al., 2014), after that, purified using hypha-tip isolation technique. The purified pathogen was stored on PDA slants at 4oC until use, (PDA) was prepared according to manufacturer's recommendation by dissolving 39g of

dehydrated PDA in 1 litter of distilled water and autoclaved at 121oC for 20 min. the medium was allowed to cool 45-50oC (Sastry and Bhat, 2018). The medium was supplemented with Streptomycin sulfate (250 mg L-1) as a bacteriostatic agent (Ismael and Mahmud, 2016).
Percentage of fungal frequency was calculated as in the following equation (Mahmood and Ismael, 2020).

$$\textbf{Fungal frequency \%} = \frac{\textbf{No.of the colonies (for each fungus)}}{\textbf{Totall No. of all fungal colonies}} \times \mathbf{100}$$

Identification of the isolated fungi

The fungi were identified by using two methods: morphologically and molecularly studied.

Morphological characteristics of the fungi

The genera and species were identified by depending on their colony morphology, color and growth habits, in addition to the appearance of the colony. To examine hyphal growth by using a Motic microscope Model 1802 LED, images captured by Microscope Digital Camera (AmScope, 18MP USB 3.0, Model MU1803) was used, the pure isolated fungi were identified by the most documented keys and images matched with fungi identification books as mentioned by (Barnett and Hunter, 1999, Elad et al., 2004, and Pitt and Hocking, 2009).

Molecular identification

In order to confirm the accuracy of morphological identification, the isolated all species were subjected to molecular analysis with fungus-specific universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). Sodium Dodecyl Sulfate (SDS) method was used for genomic DNA extraction of fungi, following the methodology previously described by Niu et al. (2008) genomic DNA of the fungi were used for PCR amplification. All amplification reactions were carried out in volumes of 50 µl containing 20 µl master mix, 4 µl of each pimer, 10 µl DNA template, and 12 µL of nuclease free water. PCR was carried out using the following condition: initial denaturation at 94°C for 5 min; 37 cycles of denaturation (94°C for 10 min., 1 cycle), 37 cycles at a melting temperature of (95°C for 1 min.), annealing (55°C for 1 min.), and extension (72°C for 2 min.); and a final extension step at (72°C for 10 min.,1 cycle) (Visagie et al., 2014).

The quality and quantity of DNA extract was checked by Nanodrop spectrophotmeter, (Nano Plus/ Maan LB., Sweden). PCR products were detected in 1% agarose stained with ethidium bromide gels in 1×TAE buffer, the electrodes of the chamber were connected to the power supply and run was achieved at 84 V for 90 min. (RUNVIEW-S, CS CLEAVER Scientific Ltd/ the UK), finally, the bands in the gel were visualized by the gel documentation system (ENDUROTM GDS Touch, LABNET). PCR amplicon was purified by the Addbio Qucik Gel Extraction Kit and sequenced by the Sanger sequencing method performed by Macrogen (South Korea), the obtained nucleotide sequences were trimmed using SnapGene ® software (GSL Biotech, Version 3.2.1, available at snapgene.com), and compared with those already stored in the National Center for Biotechnology and Information (NCBI) sequence database, using of the Basic Local Alignment Search Tool (BLAST).

PATHOGENICITY OF THE ISOLATED FUNGI

Healthy strawberry fruits in the maturity stage were used to carry out Koch's postulates. Strawberry fruits were washed and surface sterilized by (2% NaClO). Small pieces of strawberry fruits (5 mm) were taken out of the fruit. Spore mycelial slices (5 mm in diameter) were cut from seven day old cultures grown on PDA and placed in the wounds and topped with strawberry fruit fragments. Control fruits were treated the same way, but a sterile PDA disk was placed in each wound instead of mycelium. All PDA cultured grown fruits were incubated at (20-25oC) for (3-7) days until the fungi appeared. The pathogen was reisolated from lesions that developed on inoculated fruits (Chliyeh et al., 2014).

DISEASE INCIDENCE AND SEVERITY %.

The percentage of decay strawberry fruits were monthly measured, for estimating disease incidence and severity by applying the following formulas as described by (Wheeler, 1969).

$$\textbf{Incidence of disease \%} = \frac{\textbf{Number of infected fruits}}{\textbf{Number of total fruits}} \times \mathbf{100}$$

$$\textbf{Disease severity } \% = \frac{\textbf{Sum of the individual disease assessments}}{\textbf{Number of fruits observed} \times \textbf{Maximum disease quality}} \times 100$$

In addition, for assessing the disease severity% (0-5) scale were applied as described by (McKinney, 1923, and Romanazzi et al., 2013) are shown in Table 1.

Table (1) Assessing disease severity by using 0-5 scale

Severity scale	0	1	2	3	4	5
Fruit surface infected%	Healthy fruit	1–20%	21–40%	41–60%	61–80%	>81%

IN VITRO MANAGEMENT OF THE ISOLATED FUNGI.

Plant material and chemicals used in the study

Rosemary (Rosmarinus officinalis L.) leaves and pomegranate (Punica granatum L.) peel

The Rosemary leaves at (1% and 2%) concentrations, and pomegranate peel at (2% and 4%) concentrations were collected in public garden in both Sulaimani and Halabja governorates respectively. Preparation of ethanolic for the two extracts were prepared according to the method described by Balakrishnan and Kokilavani (2012).

The analysis of a certain chemical composition of plant extracts (rosemary leaves and pomegranate peel) were performed by (Shimadzu UV/ VIS spectrophotometer model 1600A-Kyoto, Japan) that are shown in Table 2, and for the others chemical composition (High Performance Liquid Chromatography HPLC Model SYKAM- Germany) were used, that are show in Table 3.

Table (2) Some chemical composition of pomegranate peel and rosemary leaves

No.	Name of tests	Pomegranate peel	Rosemary leaves	References
1	Total phenolic compound	+	+	(Zare *et al*., 2014)
2	Total Flavonoid compound	+	+	(Baba and Malik, 2015)
3	Total Alkaloid	+	+	(Ajanal *et al*., 2012)
4	Total Tannin	+	-	(Abdelkader *et al*., 2014)
5	Total Glycoside	+	+	(Tofighi *et al*., 2016)
6	Total Saponins	+	-	
(+) given by the test that was available and (-) was not available.				

Table (3) Some chemical composition of pomegranate peel and rosemary leaves

Name of plant extracts	Name of Chemical composition												References
	Apigenin	Catechine	Qurcetine	Cumarine	Keamferol	A-pinen	Camphor	Sabinen	Linalool	Limonene	Myrcene	Cineol	
Pomegranate peel %	+	+	+	+	+	-	-	-	-	-	-	-	(Hcini *et al*., 2013, Mradu *et al*., 2012)
Rosemary leaves%	+	+	+	+	+	+	+	+	+	+	+	+	
(+) given by the test that was available and (-) was not available.													

Salicylic acid ($C_7H_6O_3$) (SA) and potassium metabisulphite ($K_2S_2O_5$) (KMS)

Salicylic acid with purity of 99.9%, Alfa Aesar Company, Germany and (KMS) with purity of 99.9%, Brupaks Company, England were used by four different concentrations (100, 200, 500 and 1000 ppm) respectively.

Pristine fungicide ™ WG and Benomyl fungicide 50% Wp

Pristine and Benomyl purchased from local markets, Pristine fungicide (Pyraclostrobin 12.8% and Boscalid 25.2%, Supplement and Distribution Dabana Company for Ltd. Modern Agriculture) was used by five different concentrations (20, 50, 100, 200, and 300 ppm) and

Benomyl fungicide produced by Hockly international Ltd, USA was used at (100, 200 and 300 ppm) concentrations.

The effect of plant extracts, chemicals, and fungicides on mycelium growth inhibition rate of the fungi

Poison Food Technique (PFT) (Dixit *et al*., 1976, and Hassan *et al.*, 2014) also used to determine antimycotic activities of those materials as mentioned earlier.

Subsequently, 7 mm diameter mycelium disc was cut from seven day old pure culture of the fungi using sterile cork borer and placed face down on the center of the solidified medium-extract mixture.The inoculated plates were incubated at 25 ± 2°C. The fungi growth were monitored daily until the fungi in control plate reached the edge of the petri plates, radial growth of the fungi were measured by ruler in two different angles at 90^{o} to each other and the mean calculated (Nashwa and Abo-Elyousr, 2013). The inhibitory effects of the extracts and chemical were calculated (Smith *et al.*, 2009, and Oraki *et al*., 2011) on the 5th and 10th day as following the equation below:

$$\mathbf{MGI\%} = \frac{\mathbf{Dc-Dt}}{\mathbf{Dc}} \times \mathbf{100}$$

Where: MGI% = percentage of mycelial growth inhibition, Dc = diameter of control, Dt = diameter of mycelial growth inhibition.

STATISTICAL ANALYSIS

The percentage of disease incidence and severity laid out according to factorial Randomized Completely Block Design (RCBD), and for in vitro management of the isolated fungi laid out according to Completely Randomized Design (CRD) with three replicates, the the analysis of variance (ANOVA) was carried out using Statistical Program from Statistics and Graphics Guide (Addinsoft, 2016). Mean values were separated based on Duncan's at ≤ 0.05 probability levels (Al-Rawi and Khalafullah, 1980).

RESULTS AND DISCUSSION

Morphological Characteristic of Isolated Fungi

The microscopic characteristics studied and observed according to the literatures were mentioned in the materials and methods. the nine species were identified as *Alternaria alternata* (Figure 1 A), *Aspergillus niger* (Figure1 B), *Botrytis cinerea* (Figure 1 C), *Cladosporium cladosporioides* (Figure 1 D), *Fusarium oxysporum* f. sp. *fragariae* (Figure1 E), *Nigrospora sphaerica* (Figure 1 F), *Penicillium griseofulvum* (Figure 1 G), *Penicillium raistrickii* (Figure 1 H) and *Rhizopus stolonifer* (Figure 1 I)

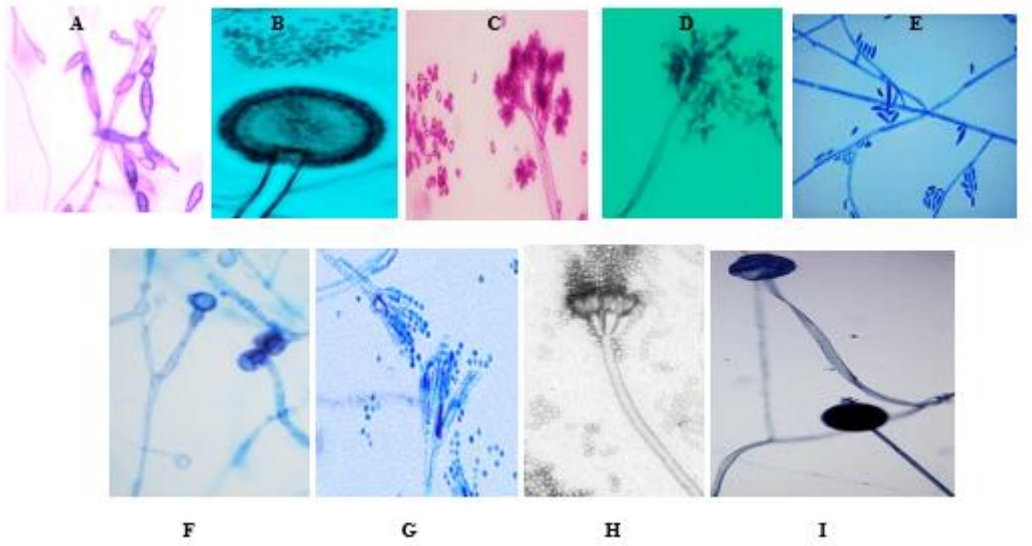


Figure1. The pathogenic fungi isolated of strawberry fruits collected from local markets observed under light microscope (Motic microscope Model 1802 LED), images captured by Microscope Digital Camera (AmScope, 18MP USB 3.0, Model MU1803), by using (100x and 400x) magnification, grown on potato dextrose agar (PDA) medium (pH = 5.4 – 5.8) at 25±2oC as the ecophysiological conditions, where Alternaria alternata (A), Aspergillus niger (B), Botrytis cinerea (C), Cladosporium cladosporioides (D),

Fusarium oxysporum f. sp. fragariae (E), Nigrospora sphaerica (F), Penicillium griseofulvum (G), Penicillium raistrickii (H) and Rhizopus stolonifer (I)

MOLECULAR IDENTIFICATION OF THE FUNGI

The molecular techniques using PCR was achieved for nine of the fungi as showed in Table 4 and using the universal primers ITS1 and ITS4 (White *et al*., 1990). After trimmed and aligned, the identification of the species were determined based on the best score, the total size of the ITS1 and ITS4 regions, including the 5.8S rDNA gene of the isolates studied varied from 492 to 725 bp, also the results showed that the sequence of the extracted isolates had the highest similarity between 97 to 100%, the PCR product of the fungi ITS region on gel electrophoresis appear as ~ 540 pb band size that are shown in Fig.2 and 3, these results are in accordance with the finding of Woo *et al*. (2010) *R. stolonifer*, (Behr *et al*., 2013) *B. cinerea*, (Bensch *et al*., 2012) *C. cladosporioides*, (Singha *et al*., 2016) *F. oxysporum* f. sp. *fragariae* (Mohammadi and Bahramikia, 2019) *A. alternata*, (Zhao *et al*., 2012) *N. sphaerica*, (Henry *et al*., 2000) *A niger*, and (Visagie *et al*., 2014, Banani *et al*., 2016) *P raistrickii*, and *P griseofulvum* respectively.

Table (4) shows nine fungal accession numbers, and BLAST identity

No.	Identification verified by sequence BLAST, (BLAST identity)	Max. Score	Total Score	Query Cover	E value	BLAST identity (%)	GenBank, Accession number	base pair (bp) size of band from Sequencing
1	*Alternaria alternate*	941	946	100%	0.00	100%	MT089993.1	509
2	*Aspergillus niger*	1162	1473	99%	0.00	98%	MF078659.1	667
3	*Botrytis cinerea*	1277	2097	99%	0.00	97%	KP749184.1	701
4	*Cladosporium cladosporioides*	904	1200	100%	0.00	99%	MK761055.1	495
5	*Fusarium oxysporum*	1194	1194	99%	0.00	98%	KX456097.1	687
6	*Nigrospora sphaerica*	904	915	100%	0.00	99%	MH619724.1	492
7	*Penicillium griseofulvum*	1042	1752	100%	0.00	97%	MF034654.1	632
8	*Penicillium raistrickii*	979	1132	97%	0.00	99%	KX056232.1	646
9	*Rhizopus stolonifer*	1286	1286	100%	0.00	99%	KC291246.1	725

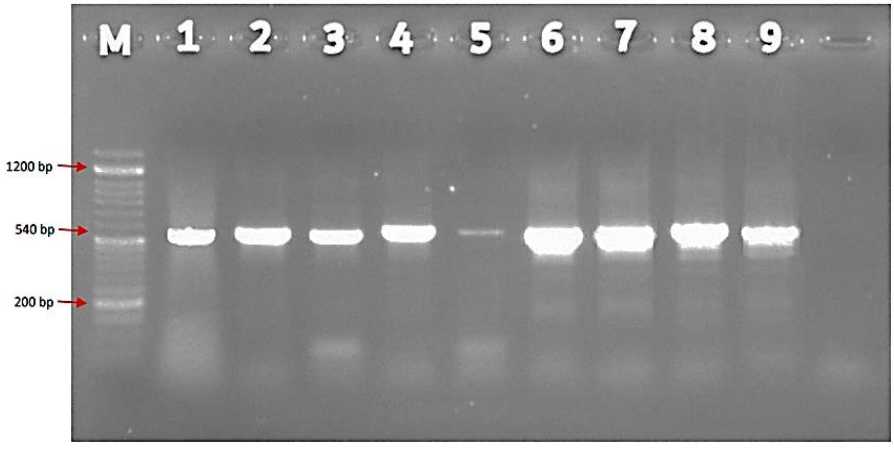


Figure 2. Agarose gel showing PCR product (540bp) of ITS1 and ITS4 DNA. Lanes: (M) DNA Lader (100 bp), L1: *R. stolonifer*; 2. N. *sphaerica*; 3. *B. cinerea*; 4. *A. alternata*; 5. *F. oxysporum*; 6. *C. cladosporioides*; 7. *P. raistrickii*; 8. *P. griseofulvum* and 9. *A. niger*

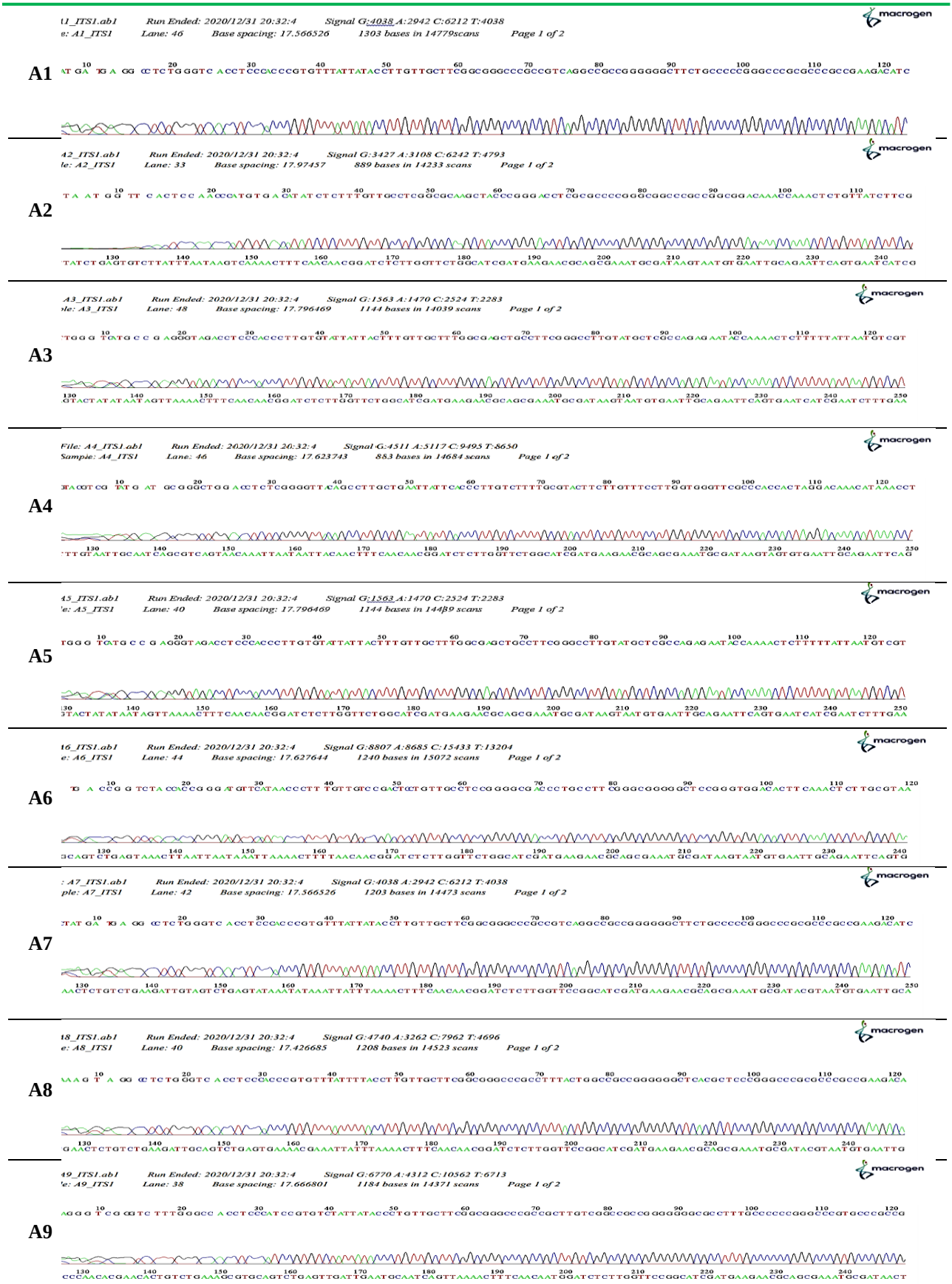
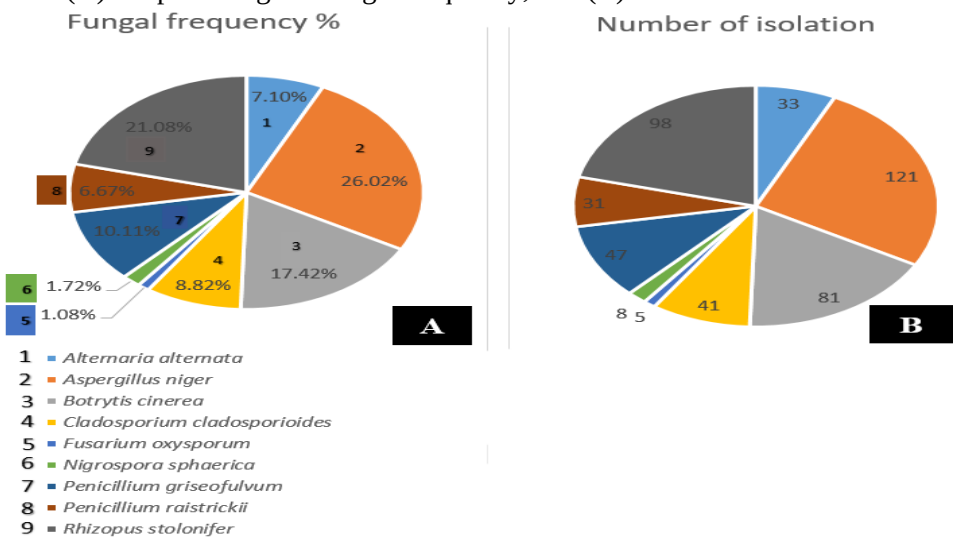


Figure 3. Partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer2, where A1: R. stolonifer; A2. N. sphaerica; A3. B. cinerea; A4. A. alternata; A5. F. oxysporum; A6. C. cladosporioides; A7. P. raistrickii; A8. P. griseofulvum and A9. A. niger

FREQUENCY PERCENTAGE OF THE FUNGI ISOLATED FROM STRAWBERRY FRUITS

Isolation of the causal agent the fungi attacking strawberry fruit, nine fungal species from 465 colonies were achieved as shown in Fig. 4, percentage of fungal frequency resulted as *A. niger*, which record 26.02 % followed by *R. stolonifer* 21.08%, *Botrytis cinerea* 17.42%, *P. griseofulvum* 10.11 %. Fungal frequency was also found to be less than 10%, such as *P. raistrickii*, *A. alternata*, *C. cladosporioides*, *N. sphaerica* and were recorded, while less fungal frequency 1.08% from *F. oxysporum* was observed on fruits. Survey of fruits markets and recognition of major post-harvest pathogens of strawberry fruits in Sulaimani Governorate, is being reported for the first time in the present investigation, this study focused on the isolation and identification of fungi on strawberry fruits in various local markets, causing decay and financial losses.

Figure 4. (A) the percentage of fungal frequency, and (B) number of isolations causing strawberry fruit rots from local markets in Sulaimani Governorate.



Strawberry (*Fragaria ananassa*), one of a major crop worldwide and of high value crops, are exposed to several infectious diseases. These results are consistent with the finding (Embaby *et al*., 2016 and Javeed and Fatima, 2017) presented that isolation of the causal agent for fungi attacking strawberry fruits yielded three fungal genera, including (*B. cinerea, R. stolonifer, Alternaria* sp., *Aspergillus* spp., *and Penicillium* spp.) are the most fungal isolates that cause strawberry fruit rates, also decayed strawberry fruits caused by *F. oxysporuim* f. sp. *fragariae*.

DETERMINATION OF PATHOGENICITY OF ISOLATES

Characteristic symptoms of disease occurred after 3-7 days of inoculation on the ripe fruits to nine fungus examined in this study. The difference in disease incidence of infection caused by the nine fungal isolates on the ripe fruits were highly significant injured fruits. The non-inoculated control fruits, the injured ones did not develop decay symptoms. In contrast, all the wounded fruits developed rot and decay, regardless of the isolates used. In particular, the isolates of all fungi observed after inoculation on the wounded fruits showed the fruit infection more than 90%, for instnace; isolate *R. stolonifer* showed the maximum value of disease incidences by 100% ,while the isolate *N. sphaerica* observed the minimum value of the disease incidence 90.83% that are shown in Fig. 5. In addition, control fruits showed no symptoms of the disease and remained healthy throughout the experiment. In advanced stage of the disease. The re-isolation from artificially infected tissue on the PDA consistently yielded all fungi, thus fulfilled Koch's postulate. There was a smiliar results with previous report by Zhang *et al.* (2019).

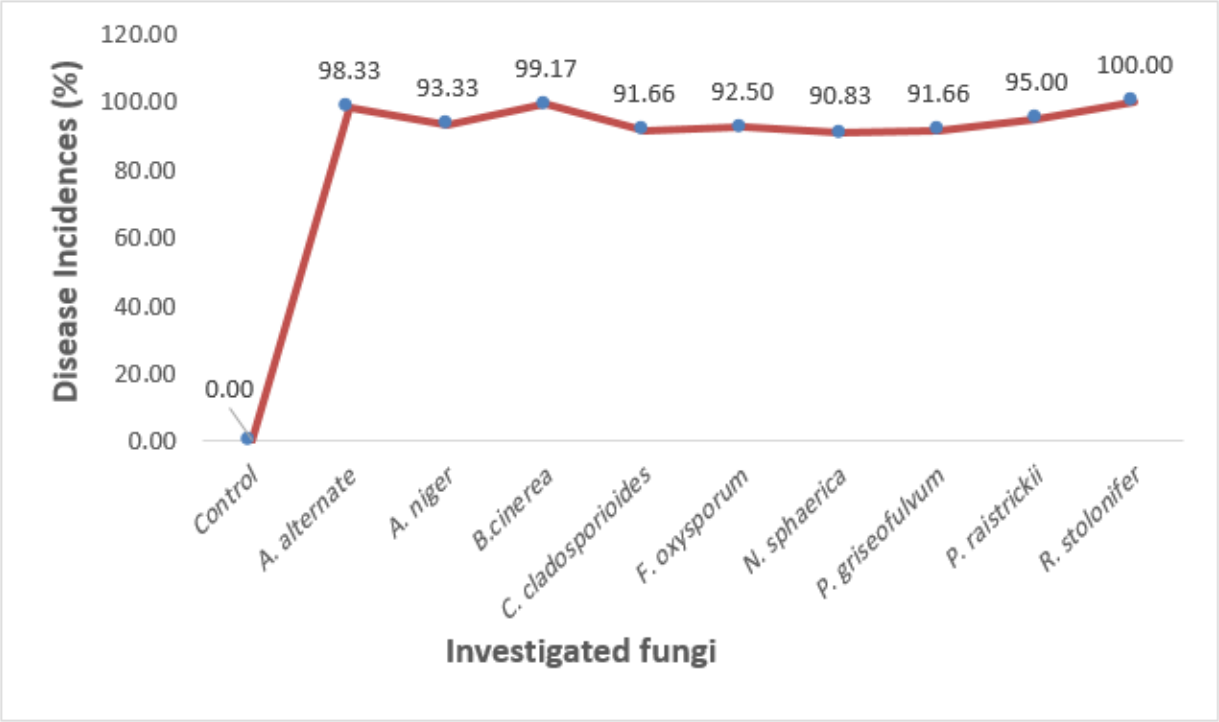


Figure 5. The pathogenicity of the nine of fungal isolates after inoculation onto the postharvest strawberry fruits

DISEASE INCIDENCE AND SEVERITY % OF THE FUNGI FRUITS

Data presented in Tables 5 and 6 explain the incidence and severity % from April to July 2019. Due to the effect of sources of strawberry fruits, no significant differences were observed. On the other hand, significant differences from the period (April to July 2019) were confirmed that the highest value was recorded with incidence 15.788% and severity 6.651% in April, respectively, while the lowest value was observed 10.340% and 4.353% in July. In addition, to the interaction effect between sources of strawberry fruits with the period, significant differences were noticed that the highest value of the incidence and severity were registered on strawberry fruits imported from Turkey in April 18.773% and 4.348% respectively. Whereas, the strawberry imported from Iran in July, gave smallest value of the incidence 7.737% and severity 4.348%. There are few reliable data on the prevalence and disease incidence and severity of strawberries according to estimates of the harvest at the wholesale markets of Sulaimani Governorate. A significant proportion loss of fruit and vegetables after harvest is a matter of great economic concern in agriculture. Loss after harvest no only effect on qualitative and quantitative of fruits, but also has negative influences on human health due to fungal mycotoxin risks, which became inedible and palatable by the consumer (Adeoye *et al*., 2009 and Buyukbay *et al.*, 2011). The high incidence of post-harvest damage means that production of strawberry fruit technology in Sulaimani Governorate needs improvements to deliver good quality fruit. Enormous amount of fruit and vegetables are wasted every day due to the lack of suitable facilities after harvest in the fruit and vegetable market, cooling perishable foods is the most convenient way to delay chemical, biological processes and growth of microorganisms, because the fruit can remain on the wholesale market for a longer time and then be transported to the place where it is marketed at room temperature, (Devkota *et al*., 2014). The amount of losses caused by post-harvest losses in the Kurdistan province of Iran was recorded on strawberry, where the total production was about 22679 ton, the average strawberry loss after harvest turned out to be 28% and estimated at 6350.12 ton, also the total strawberry loss after harvest was equal to 7,809,200$ (Salami *et al.*, 2010). There are insufficient processing factories in the growing regions. As strawberries are very perishable, the transport system is not well equipped to deliver fresh fruit to distant markets. Therefore, prices in the production season usually fall sharply (Eshghi *et al*., 2007).

Table (5) Disease incidence (%) of strawberry fruits imported from Turkey and Iran which sold in local markets in Sulaimani Governorate durig (April to July 2019)

Imported source of Strawberries fruit	Month				Main effect source of strawberry fruits
	April	May	June	July	
Turkey	18.773 a	7.916 c	11.583 bc	12.942 bc	12.803 a
Iran	12.803 bc	13.916 ab	12.916 bc	7.737 c	11.843 a
Main of month effect	15.788 a	10.916 b	12.250 b	10.340 b	

*Means with the same letters are not different significantly by Duncan's Multiple range test ($P \leq 0.05$).

Table (6) Disease severity (%) of strawberry fruits imported from Turkey and Iran which sold in local markets in Sulaimani Governorate durig (April to July 2019)

Imported source of Strawberries fruit	Month				Main effect source of strawberry fruits
	April	May	June	July	
Turkey	7.873 a	4.388 c	5.099 bc	4.359 c	5.430 a
Iran	5.430 bc	6.727 ab	4.913 c	4.348 c	5.355 a
Main of month effect	6.651 a	5.558 ab	5.006 b	4.353 b	

*Means with the same letters are not different significantly by Duncan's Multiple range test ($P \leq 0.05$).

THE EFFECT OF PLANT EXTRACTS, CHEMICALS, AND FUNGICIDES ON MYCELIUM GROWTH INHIBITION RATE OF THE FUNGI

Results were expressed as efficacy of plant extracts, chemicals and fungicides (rate of inhibition of mycelial growth compared to untreated control). The mycelial growth of the 9 of fungal isolates has been affected differently by the 21 treatments which are shown in Table 7. The highest mycelial growth was 100% against all isolated fungi which were achieved with salicylic acid at concentrations 1000 ppm, followed by 500 ppm, had the same effect in all fungi except of *F. oxysporum*. In addition, SA exhibited superior inhibitory effect on the growth of *P. raistrickii* at 200 ppm, SA is gaining importance in maintaining postharvest quality of fruits, is reported to be directly toxic to fungi as it significantly inhibits fungal growth and spore germination of the pathogen in vitro (Jyoti, 2015), these results are similar with the findings of other authors, such as (Wang *et al*., 2011 and Zhang *et al*., 2008) who found that antifungal activity of SA was detected against several postharvest pathogens, including *B. cinerea* and *F. oxysporum* (Qin *et al*., 2015) and *A. alternata* (Ismae and Omer, 2018) found that MIR reached 100% when salicylic acid was used at 500 ppm. In the same Table 7, the results revealed that potassium metabisulphite had the best result in the concentration 1000 ppm than 500 on eight of fungi except of *R. stolonifer*, these results were agreement with the other researcher (Kayraldiz *et al.*, 2006).

Table 7 shows that the biological activity of plant extracts (*R. officinalis* and *P. granatum*) had no antifungal properties against seven fungi in all the concentrated levels, and there were no significant differences at 1 and 2% concentrations *R. officinallis* and (2 and 4% concentrations) *Punica granatum*. On the other hand *R. officinalis* extracts at different concentrations had the best effect on *P. griseofulvum* for both concentrations, which mentioned in the same table and *P. granatum* peel 4% on *N. sphaerica* whereas the inhibition growth of mycelia 100% was recorded. Different secondary metabolite containing different compounds including (flavonoids, phenolic compounds, tannins, saponins, alkaloid and glycosides) isolated from pomegranate peel and rosemary leaf extract, which had measurable influences on the inhibition of the fungi. The fungicidal activity of pomegranate extract against some phytopathogenic fungi has been well reported by Dahham *et al.* (2010) who tested different pomegranate extracts on linear growth of different fungi, they found that the highest fungicidal activity was recorded on *A. niger* followed by *Penicillum* sp. and *Rhizopus* sp. based on spectral analysis, the compound of pomegranate reindeer extract exhibiting strong antimycotic activity, Endo *et al.* (2010) also reported that

pomegranate extract had different antifungal metabolites compounds such as punicalagin and castagalagin. Rongai *et al*. (2019) investigated the antifungal activity of 21 different pomegranate genotypes, aquatic and ethanolic extracts which had significantly affected on the tested on fungi.

Furthermore, Lattanzio *et al*. (2006) revealed that the pomegranate peel extract containing various compounds including flavonoids, phenolic compounds, tannins and glycosides, these compounds were reported to have in vitro antimicrobial activities on *B. cinerea, A. niger*, and *Penicillum*

The essential oil of the rosemary plant has been studied all over the world, the chemical compounds, antimicrobial and antifungal effects of the rosemary plant have been studied (Angioni *et al*., 2004).

Pristine fungicide at the concentration of 20, 50, 100, 200, 300 ppm had remarkable effects on the fungi. They were inhabited mycelial growth rate to 100% exactly *on C. cladosporioides, P. raistrickii, N. sphaerica, A. niger* and *p. griseofulvum* the whole concentration. Some researchers pointed out the similar results obtained in an experiment that using fungicide treatment had the inhibitory effect on mycelial growth at different concentrations against many plant pathogenic fungi (Gudmestad *et al*., 2013 and Avenot *et al.*, 2008) registered for control of fungus (*A. alternata)* Both pyraclostrobin and boscalid provided satisfactory control of *B. cinerea* (Myresiotis *et al*., 2008).

Benomyl at the concentration 100, 200 and 300 ppm were also inhibited 100% of all tested fungi mentioned in the previous section by the addition of *f. oxysporum,* while the lowest concentration 20 ppm of Pristine, and 100 ppm Benomyl had no significant effects on *Rhizopus stolonifer and Botrytis cinerea*. In all cases, the mycelial growth of all fungi were completely stopped, very close to at low and high concentration of (chemicals, fungicides and plant extracts) which were used.

HPLC and Spectrophotometer Analysis of Some Chemical Composition of Plant Extracts (Rosemary Leaves and Pomegranate Peel)

Table 8 shows some chemical composition of (rosemary leaves and pomegranate peel) plant extracts by UV/VIS spectrophotometer, the amount of total phenolic compound (%), total flavonoid compound (%) and total glycoside (%) in *P. granatum* was recorded more than in *R. officinalis* whereas the value 13.76, 7.76 and 8.41% in *P. granatum* and 6.66, 3.33 and 6.88% in *R. officinalis* were documented, respectively. Furthermore, total alkaloid in *R. officinalis* 21.58% was shown more than in *P. granatum* 13.59%.

HPLC Analysis of Some Chemical Composition of Plant Extracts (Rosemary Leaves and Pomegranate Peel)

Table 9 shows 12 volatile components were identified on the basis of their (HPLC) properties., the maximum (%) of chemical composition in rosemary was Limonene 14.9% while the minimum (%) was Cineol 2.5%, on the other hand the maximum phenolic compound in *P. granatum* peel was Qurcetine 48.9% while the minimum percentage was Catechine 12.58% Thus, the amount of phenolic compound with pomegranate peel was better than the rosemary leaves. This result is consistent with Elfalleh *et al*. (2012) and Li *et al.* (2006)) the total polyphenol content (TPP) is highest in peel pomegranate, Pande and Akoh (2009) also reported that total polyphenol in peel was more than in seeds and leaves of Georgian pomegranate.

Table (7) The effect of plant extracts, chemicals, and fungicides on mycelium growth inhibition rate of the fungi

Treatments	Concentrations	*R. stolonifer*	*B. cinerea*	*C. cladosporioides*	*P.raistrickii*	*F. oxysporum* fof	*A. alternata*	*N. sphaerica*	*A niger*	*P. griseofulvum*
Control	0	0.000 i	0.000 i	0.000 f	0.000 f	0.000 i	0.000 h	0.000 e	0.000 g	0.000 e
Rosemary leaves extract	1%	0.000 i	25.364 g	12.069 e	45.741 c	42.216 g	51.296 de	38.704 c	24.652 f	100.000 a
	2%	0.000 i	34.808 ef	34.208 d	60.185 b	59.645 cd	70.926 c	80.556 b	48.102 d	100.000 a
pomegranate peel extract	2%	0.000 i	29.630 fg	12.040 e	21.111 e	44.382 fg	53.889 d	41.852 c	33.557 e	39.815 d
	4%	0.000 i	44.667 d	43.171 d	45.000 c	57.548 cd	56.481 d	100.000 a	51.143 cd	50.000 c
Salicylic acid	100 ppm	0.000 i	29.815 fg	35.943 d	32.407 d	39.036 g	54.815 d	19.444 d	28.704 f	48.333 c
	200 ppm	0.000 i	52.222 c	82.694 b	100.000 a	58.436 cd	83.333 b	40.556 c	54.444 c	86.667 b
	500 ppm	100.000 a	100.000 a	100.000 a	100.000 a	67.534 b	100.000 a	100.000 a	100.000 a	100.000 a
	1000 ppm	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a
Potassium metabisulphite	100 ppm	0.000 i	57.500 c	38.215 d	36.111 d	31.905 h	56.852 d	100.000 a	61.111 b	100.000 a
	200 ppm	0.000 i	86.667 b	63.089 c	100.000 a	50.697 ef	71.111 c	100.000 a	63.611 b	100.000 a
	500 ppm	36.352 g	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a
	1000 ppm	95.741 b	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a	100.000 a
Pristine	20 ppm	0.000 i	0.000 i	100.000 a	100.000 a	49.354 ef	56.481 d	100.000 a	100.000 a	100.000 a
	50 ppm	44.296 f	0.000 i	100.000 a	100.000 a	53.342 de	71.111 c	100.000 a	100.000 a	100.000 a
	100 ppm	56.222 e	29.815 fg	100.000 a	100.000 a	60.976 c	76.296 c	100.000 a	100.000 a	100.000 a
	200 ppm	73.056 d	54.259 c	100.000 a	100.000 a	69.237 b	82.407 b	100.000 a	100.000 a	100.000 a
	300 ppm	81.389 c	56.389 c	100.000 a	100.000 a	71.771 b	83.333 b	100.000 a	100.000 a	100.000 a
Benomyl	100 ppm	0.000 i	0.000 i	100.000 a	100.000 a	100.000 a	31.944 g	100.000 a	100.000 a	100.000 a
	200 ppm	0.000 i	13.944 h	100.000 a	100.000 a	100.000 a	38.704 f	100.000 a	100.000 a	100.000 a
	300 ppm	14.090 h	38.333 e	100.000 a	100.000 a	100.000 a	48.333 e	100.000 a	100.000 a	100.000 a

* Means with the same letters are not different significantly by Duncan's Multiple range test ($P \leq 0.05$)

Table (8) Some chemical composition of pomegranate peel and rosemary leaves

No.	Name of tests	Pomegranate peel %	Rosemary leaves%
1	Total phenolic compound	13.76	6.66
2	Total Flavonoid compound	7.76	4.33
3	Total Alkaloid	13.59	21.58
4	Total Tannin	23.89	-
5	Total Glycoside	8.41	6.88
6	Total Saponins	2.36	-
(-) given by the test that was not available.			

Table (9) Some chemical composition of pomegranate peel and rosemary leaves

Name of plant extracts	Name of chemical composition											
	Apigenin	Catechine	Qurcetine	Cumarine	Keamferol	A-pinen	Camphor	Sabinen	Linalool	**Limonene**	**Myrcene**	**Cineol**
Pomegranate peel %	32.5	12.58	48.9	20.3	22.5	-	-	-	-	-	-	-
Rosemary leaves%	12.3	6.5	12.5	13.5	8.9	13.8	8.9	4.8	9	14.9	5.8	2.5
(-) given by the test that was not available.												

CONCLUSION

Biological control agents and bio-products are representing environment-friendly agents for strawberry integrated protection against diseases. Technological tools lead to minimizing chemical efforts in integrated control of strawberry diseases. Based on the present study, it may be concluded that plant extracts *Rosmarinus officinalis* L., and *Punica granatum* L. could be an effective bio-control agent against strawberry fruit decay caused by some common fungi such as *Penicillium griseofulvum* and *Nigrospora sphaerica*. The Results showed that SA and KMS exhibit a high potential in controlling postharvest diseases of strawberry fruits in vitro study. We propose the following as a starting point: fruit sales in markets should take place in a clean and safe environment that is not good for the growth of fungi. It is recommended to improve technology-based preservation techniques be used to improve the fruit's keeping quality.

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تحديد وتشخيص الفطريات المصاحبة لثمار الفراولة المأخوذة من الاسواق المحلية في السليمانية وتقنيات إدارتها مختبريا

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الخلاصة

الكلمات المفتاحية: الشليك ، الفطريات، تردد الفطريات، المستخلصات النباتية والمواد الكيماوية.

جمعت عينات من ثمار الشليك من ألاسواق المحلية في محافظة السليمانية-العراق مستوردة من إيران وتركيا بهدف تحديد وتشخيص الفطريات المصاحبة لها. حيث تم استخدام طريقتين مختلفتين للتشخيص؛ ففي الطريقة الاولى تم اتباع الخصائص المظهرية المورفولوجية مثل شكل السبورات واللون والشكل العام للفطر، وأيضًا تم تأكيد التشخيص باتباع طريقة البايولوجيا الجزيئية ، حيث تم تشخيص تسعة فطريات مختلفة بالاعتماد على نتائج البيانات الجزئية NCBI-BLAST لمعدل تعرف بين 97-100%. تم تشخيص الفطريات الاتية: Alternaria alternate، Aspergillus niger، Botrytis cinerea، Cladosporium cladosporioides، Fusaruim oxysporum f. sp. fragariae، Nigrospora sphaerica، Penicillium raistrickii، Penicillim griseofulvum و Rizhopus stolonifer.سجلت اعلى نسبة اصابة لثمار الشليك بالفطر A. niger بنسبة 26.02%،

بينما سجلت أقل نسبة إصابة بالفطر F. oxysporum بنسبة 1.08%، تم إجراء اختبار الإمراضية للفطريات المعزولة وكشفت النتائج أن جميع الفطريات ذات قابلية امراضية عالية بأكثر من 90%. لم تظهر العينات المأخوذة من الأسواق المحلية أي فروقات معنوية في معدل الإصابة بالمرض وشدة المرض. لإدارة الفطريات المصاحبة للشليك، تم استخدام المستخلصات النباتية إكليل الجبل والرمان وحمض الساليسيليك SAوميتا بيكبريتيت االبوتاسيوم KMSواثنين من مبيدات الفطريات .1% و 2% مستخلص إكليل الجبل أعطت أعلى نسبة تثبيط P. griseofulvum في حين أن مستخلص قشور الرمان 4% له تأثير معنوي على N. sphaerica بالإضافة إلى ذلك، كانت لـ SA و KMS فاعلية على جميع الفطريات باستثناء الفطر R. stoloniferلميتا بيكبريتيت االبوتاسيوم KMSحيث لم يظهر أي تأثير عليه و مقارنة بمعاملة المقارنة الشاهد.