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FURTHER EVIDENCE ON SYSTEMIC RESISTANCE INDUCED BY CONTAMINANT MICROBIAL IN POTATO CULTURE *IN VITRO*

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Abstract

This study is concerning with searching use of the phenomenon of systemic acquired resistance (SAR) to control plant viruses and increased growth *in vitro*. Through this study, it was and proved to induced SAR in potato plants using bacterial and fungal contaminants in tissue culture against *Potato virus Y*. These biotic inducers were *Pseudomonas* sp., *Bacillus* sp., *Xanthomonas* sp. as well as *Trichoderma*, *Aspergillus* and *Fusarium* as contaminants microbial of potato micropropagative *in vitro*. The occurrence of induced resistance and increased potato plantlets were found by MS medium treated with culture filtrates based on reduction of PVY infection, disease severity, biochemical changes accompanied to induced resistance (the high level of endogenous salicylic acid, protein content, chlorophyll contents and peroxidase and polyphenol oxidase activities as well as increasing in growth parameters).

Key words: Potato, PVY, Biotic inducers, Tissue culture, Induced resistance, PAGE

Introduction

Plant tissue culture is a tool, which allows the rapid production of many genetically identical plants using a piece of a plant such as a stem tip, node, meristem, embryo, or even a seed and placing it in a sterile nutrient medium where it multiplies. The nutrient media in which the plant tissue is cultivated is a good source of nutrient for microbial growth. These microbes adversely compete with plant tissue culture for nutrients (Kane, 2003).

Microbial contamination is one of the most serious problems

of plant cell and tissue culture. A wide range of microorganisms (filamentous fungi, yeasts, bacteria, viruses and viroids) and micro-arthropods (mites and thrips) have been identified as contaminants in plant tissue cultures (**Leifert and Cassells, 2001**).

A large number of different agents induce resistance in plants against pathogens were reported. Induced resistance is accomplished by treating plant with the culture filtrate of individual non-pathogenic isolates as well as the combination of culture filtrate were the most effective in inhibiting virus infection as a result of induced systemic resistance (**Kolase and Sawant, 2007; Megahed, 2008**).

The present work aims to screening different contaminants microbial in plant tissue cultures to select the most effective ones for inducing systemic resistance in potato cultures against *Potato virus Y in vitro*.

Materials and Methods

Plant materials:

Potato cultures *in vitro* were in initiated from *Potato virus Y* infected tuber sprouts cv. Spounta grown at Virology Greenhouse Fac. Agric. Ain Shams Univ. Potato tuber sprouts were tested against PVY using polyclonal antibodies by DAS-ELISA (**Clark and Adam, 1971**).

Potato tuber sprouts were washed with tap water, and then soaked for 20 min in 3% sodium hypochloride. After surface sterilization, explants were rinsed three times with sterilized distilled water. The explants then aseptically transferred to MS base culture medium free hormones (**Murashige and Skoog, 1962**). All cultures were incubated under cultural conditions (26 ± 2□C under 16 hrs light and 8 hrs dark for three weeks). Five sprouts were used as replicates. Potato plantlets were subcultured *in vitro* onto multiplication MS medium (**Murashige and Skoog, 1962**). The cultures were weekly examined and contamination rate was calculated for bacterial or/and fungal contaminants.

Isolation and purification of microbial contaminants:

The microbes contaminated potato cultures were isolated by inoculating them on

nutrient agar medium (Jay, 1992) and incubated at 30□C for 3 days for bacterial contaminants. As well as Acidified Potao Dextrose Agar (APHA, 1989) and incubated at 26□C for 6 days for fungal contaminants. Single colony was picked on slant tube media and stored at 4□C in a refrigerator to be used as stock for further induced resistance experiments.

Characterization and identification of microbial isolates:

Bacterial isolates were identified according to **Klement *et al.* (1990)** and **Bergey's Meanwhile (1994)** at Agric. Microbiol. Labs. Fac. Agric. Ain Shams Univ. Fungal isolates were identified using culture and microscopic morphological characters and by comparison with standards (**Mathur and Olga, 2003**) at Mycology Labs. Fac. Agric. Ain Shams Univ.

Determination of induced resistance:

Bacterial culture filtrate:

A loop full of bacteria growth from on agar slope was transferred to 10 ml tryptic Soy Broth for preparation of culture filtrate according to **Zuberer (1994).** The bacteria cell was discarded from culture by spinning at 10000 rpm for 10 min and the supernatant was used as test materials.

Fungal culture filtrate:

Identified fungi from stock culture were cultured on PDA in petri dishes and incubated at 25□C for 6 days. Hyphal growth from PDA cultures was transferred to 250 ml Erlenmeyer flasks containing 150 ml of autoclaved C2apak-Dox solution for preparation culture filtrates according to **Duncan (1973).** The fungal hyphae and spores were removed by filtering through 0.45 µm Millipore filters.

The cell free supernatants of bacteria or fungi isolates were mixed with MS medium as following: MS medium (as control), 100 ml culture filtrate per 1 L MS medium and 200 ml culture filtrate per 1 L MS medium. All potato plantlets treated with culture filtrates (5 plantlet/Jar) and 5 Jar per each treatment as replicates were incubated under culture conditions.

Assessment of induced resistance:

The level of induced resistance in potato plantlets against PVY was evaluated based on:

PVY incidence and concentration:

were assayed as the percentage of infected plantlets by DAS-ELISA according to **Clark and Adams (1971).**

Plantlets growth development: Number and length of shoot and roots as well as growth value were calculated in each treatment throughout subculture after 8 weeks according to **Ziv (1992).**

Protein content:was determined according to **Bradford (1976)** using bovine serum albumin as standard protein.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on protein patterns according to the method of **Studier (1973).**

Enzyme activities: One gram of plantlets tissue was ground with 3 ml of potassium phosphate (50 mμ and pH 7.0). The homogenates were centrifuged at 12000 rpm for 10 min under 4□C. The supernatant (crude enzyme protein) was collected and divided into 1.5 ml proteins.

Peroxidase activity was determined according to **Malik and Singh (1980)** in 200 μl of the crude enzyme with 3.5 ml phosphate buffer (pH 7.0, 0.1 M) and mixed with 100 μl guaicacol (0.251). The optical density was recorded every one min for 5 min at 470 nm.

Polyphenoloxidase activity was determined according to **Coseteng and Lee (1978)** in 200 μl of the crude enzyme with 3.5 ml phosphate buffer (pH 7.0, 0.1 M) and mixed with 2.95 ml of 20 mM catechol. The optical density was recorded every one min for 5 min at 420 nm.

Determination of photopigments: Chlorophyll a, b and carotenoids were extracted and determined according to **Anon (2000).**

Determination of salicylic acid: Total salicylic acid was extracted and quantified from potato plantlets by fluorescence HPLC

according to method of Roskin *et al.* (1989).

Results

This study was carried out to detect induced resistance phenomena via the dominant bacterial and fungal contaminants through the different stages of potato micropropagated by tissue culture *in vitro*. Homologous of tuber sprouts were taken from healthy and PVY infected potato plants cv. Spounta to use in this study.

Infestation of micropropagated potato shoots:

The tools and potato explants used in tissue culture were subjected to suitable sterilization using sodium hypochloride, Dettol and UV rays, as daily regulator intervals to culture contamination in potato culture *in vitro* could be achieved by taking proper pre propagation reservations, contaminants of micropropagated potato shoots were isolated using specific media. It was observed that fungal contaminants were higher rate rather than bacterial contaminants. This trend was observed in explants and establishment stages. While the occurrence rate of bacterial isolates was higher than that of fungal isolates.

The bacterial contaminants were assayed to major genera were mostly often found associated plants and included gram negative bacteria. The bacterial contaminants were found to be the following genera: *Pseudomonas* spp; *Agrobacterium* spp; *Bacillus* spp; *Corynebacterium* spp; *Serratia* spp; *Xanthomonas* spp; *Klebsiella* spp; *Micrococcus* spp and *Enterobacter* spp **(Table 1)**. The occurrence and frequency rate of *Pseudomonas* and *Bacillus* isolates were higher followed by *Serratia* and *Xanthomonas* whereas, the lowest frequency of bacterial isolates were *Agrobacterium*, *Klebsiella*, *Enterobacter* and *Micrococcus* **(Table 1)**.

On the other hand many fungal contaminants were isolated and identified **(Table 2)** during tissue culture produces from potato cultures. These contaminant fungal isolates were *Aspergillus niger*; *Asp. funigatus*; *Asp.* Spp; *Alternaria tenuis*; *Al.* spp; *Mucor* spp; *Rhizopus* spp; *Fusarium culmcirium*; *F.* spp; *Penicillium*

spp; *Geotrichum* spp; *Candida* spp and *Trichoderma* spp. The results in **table (2)** are worthy to mention that, the isolates of *Fusarium*, *Aspergillus* and *Penicillium* genera were the highest frequency; *Alternaria*, *Geotrichum* and *Trichoderma*, were moderate, wheras the lowest frequency of fungal isolates were *Candida*, *Mucor* and *Rhizopus* genera **(Table 2)**.

Table 1. Characteristica and frequency of bacterial contaminants isolates of potato culturs.

Bacterial contaminants	Characteristics					
	Cell morphology	Motility	Gram stain	Endospore formation	Pigment production	Frequency (%)
Pseudomonas	Short rod	+	G-	No	Water soluble greenish yellow	28.56
Xanthomonas	Short rod	+	G-	No	Pale yellow	17.75
Micrococcus	Spherical	-	G+	No	Rose color inside cell	3.6
Bacillus	Long rod	+	G+	Intermediate spore	Creamy	23.75
Serratia	Short rod	+	G-	No	Dark red inside cell	10.50
Klebsiella	Short rod	-	G-	No	Creamy	4.5
Corynebacterium	Short rod irregulatory	-	G-	No	Creamy	2.25
Agrobacterium	Short rod	+	G-	No	Creamy	2.15
Enterobacter	Short rod	+	G-	No	Creamy	2.00

G-: Gram stain negative G+: Gram stain positive

Table 2. Characteristics and frequency of fungal contaminants isolates of potato micropropagation..

Fungal isolates	Colony shape	Characteristics of the genus	Frequency (%)
Aspergillus spp.	Fuzzy black color	Mycelium separated, long conidiophore and double well which has a foot cell vesicle, head carrying primary and secondary sterigmata which carry chains of conidia spores, spherical conidium and dark yellowish.	16.00
Alternaria spp.	Fuzzy black color	Mycelium separated, short conidiophore carry chains of conidia spores, conidia are large and multicellular, longitudinal; transverse septa conidia usually in chains and sometimes present single and dark yellowish.	10.50
Trichoderma spp.	Fuzzy greenish color	Mycelium separated, septate conidiophore sterile at the tip, conidia ellipsoidal tuberculate, transverse septa conidia usually in chains and sometimes present bears and yellow green pigment.	20.75
Penicillium spp.	Fuzzy greenish color	Mycelium separated, conidiophore is branched to form a broom like (brush). The multiple branching of conidiophore ends with sterigmata which bear the chains of conidia, spherical conidia with yellow color	12.75
Fusarium spp.	Fuzzy reddish color inside cell	Mycelium separated produce reddish pigments in the medium produces long crescent-shaped multiseptated microconidia andmacroconidia and very small spherical, oval elongated or crescent-shaped microconidia on simple or branched single hyphae	24.5
Rhizopus spp.	cottony	Mycelium not separated, three types of mycelium rhizoids stolons and a real sporangiophore ends with columella and spherical and black sporangiospores	5.5
Mucor spp.	cottony	As *Rhizopus* but rhizoids are not present at the base of sporangiophore	4.25
Candida spp.	Platen	Yeast-like fungal sometimes produce pseudomycelium reproduce by budding oval cells	4.25

Induced resistance in potato tissue *in vitro*:

Six biotic inducers include three bacterial and three fungal filtrates as well as microbial contaminants were used for induced resistance. Induced resistance was detected via potato plantlets growth rate, PVY infectivity and phytochemistry.

The screening of microbial contaminants for biotic inducers based on reduction of PVY infectivity and potato plantlets growth rates. Most of these fungal and bacterial contaminants have been reported to increase culture mortality and presence of latent infections can result in variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting. But, some showed obvious effect on growth parameters of potato plantlets, so two of both bacterial and fungal contaminants were chosen to study the effect of their culture filtrate on plantlets growth under tissue culture conditions.

Virus infectivity:

Potato virus Y (PVY) isolate was detected in potato sprouts using specific polyclonal antibodies by DAS-ELISA. The microbial contaminants were reduced potential of PVY infectivity in potato plantlets with different rates (**Table 3**) whereas individual contaminant gave high reduction in PVY infection and increasing when applied increase microbial filtrates concentrations.

Average of five jars containing 15-20 plantlets $^{-1}$jar. The microbial contaminants due to reduction of PVY infectivity whereas *Pseudomonas*, *Bacillus*, *Fusarium* and *Trichoderma* revealed the highest reduction of PVY infectivity (41.5, 37.5, 35.0 and 31.0 at 100 $^{-1}$L MS medium, respectively) and 45.5, 40.0, 40.5 and 35.5 at 200 $^{-1}$L MS medium, respectively. While other either treatments have lowest reduction of PVY infectivity (**Table 3**). Treated potato plantlets with microbial contaminants due to higher reduction of PVY concentration (**Table 3**) compared with individual contaminants microbial.

Table 3. Effect of individual microbial contaminants on PVY infectivity in potato plantlets.

Parameters / Microbial contaminants	100 MF $^{-1}$L MS medium			200 MF $^{-1}$L MS medium		
	Optical density	% of PVY infectivity	Reduction infection (%)	Optical density	% of PVY infectivity	Reduction infection (%)
Pseudomonas		51.5	41.5		50.25	45.5
Xanthomonas		78.0	25.0		70.50	35.5
Bacillus		55.0	37.5		60.25	40.0
Trichoderma		61.0	35.0		57.0	40.5
Aspergillus		70.0	28.0		65.5	30.75
Fusarium		78.5	31.0		70.0	35.5
Microbial contaminants		68.0	40.0		60.0	35.5

Plantlet PVY infected (+ve)=
Plantlet health (-ve)=

$$\text{Reduction infectivity (R1)} = \frac{\text{Total plantlets} - \text{treatment}}{\text{Total plantlets}} \times 100$$

Potato plantlets growth rates:

Results in table (4) showed that, natural microbial contaminants of the potato cultures or microbial filtrates of bacteria or fungi to culture medium caused increasing enhancement in all growth parameters rather than control (healthy or PVY-infected potato plantlets).

As regard to the effect of bacterial culture filtrates on shoot and roots length as well as growth rate; the results illustrated the application of bacterial filtrate at of 100 ml/L MS medium due to the increasing the highest shoot

and roots length as well as growth value (**Table 4**). Since, they were 7.75, 8.00 cm and 2.25 respectively in case of *Pseudomonas* sp, where they 7.5, 7.15 cm and 1.82 respectively in case of *Bacillus* sp and 6.5, 7.10 cm and 1.78 respectively in case of *Xanthomonas* filtrate compared with healthy ones. As well as, it is worthy to mention that the filtrates of *Pseudomonas* culture showed higher promotion effect on potato plantlets growth value in comparison with healthy and PVY-infected ones with addition of *Xanthomonas* and *Bacillus* filtrates.

Regarding the effect of fungal culture filtrates application to the MS medium obtained data in **table (4)** showed that, the fungal filtrates enhanced all growth parameters compared with control ones (without fungal filtrates application). The enhancement was increased with increasing of fungal filtrate application.

The data presented in **table (4)** showed that, *Fusarium* filtrate was more efficient on the enhancement of potato plantlets growth in tissue culture than the filtrate of *Aspergillus* and *Trichoderma*. Concerning the effect of fungal culture filtrate on shoot and roots length as well as growth value, the results revealed that the highest records of shoot and roots length as well as growth value were obtained with culture filtrate 100 ml/L MS medium. Moreover, it is worthy to mention that the filtrate of *Fusarium* culture shoed higher promotion effect on potato plantlets length and growth value compared to the filtrate of other fungal cultures.

As regard to the effect of bacterial and fungal contaminants on shoot and roots length obtained data in **table (4)** showed that the highest shoot and roots length were obtained from naturally contamined MS medium in jars since they were 7.75 and 7.20 cm respectively as well as leaves numbers 5.15 compared to control (healthy and PVY-infected no contamination).

Table 4. Effect of microbial contaminants culture filtrates on potato plantlets growth infected with PVY *in vitro*.

	Shoot length (cm)	Shoot number	Leaves number	Root length (cm)	Root number	Growth value
Healthy control (disinfectants)	6.35	1.50	4.12	6.75	7.25	1.75
Infected control (disinfectants)	6.10	1.25	4.20	5.25	6.14	1.43
Mixed microbial contaminants	7.25	1.75	4.25	6.20	7.25	1.95
Pseudomonas	7.75	2.22	5.04	8.00	8.75	2.25
Xanthomonas	6.50	1.70	4.25	7.10	6.75	1.78
Bacillus	7.50	1.75	4.70	7.15	7.41	1.82
Trichoderma	7.92	1.85	4.54	7.85	7.75	2.01
Aspergillus	8.25	2.25	4.45	8.45	9.25	2.40
Fusarium	8.60	2.30	5.25	8.75	9.50	2.60
Mixed microbial isolates filtrates	7.75	2.40	5.15	7.20	8.21	2.50

Calculated as average from 100 potato plantlets (10 jars)

Phytochemical contents:

Protein content and enzyme activities were determined in treated potato plantlets with individual and mixed microbial contaminants *in vitro*. Data in **table (5)** revealed that, all

individual microbial inducers as well as microbial contaminants increased in protein content and enzyme activities of potato plantlets *in vitro*. The highest protein content was produced by individual microbial inducers, such as *Bacillus* (1.75); *Aspergillus* (1.80); *Fusarium* (1.70) followed by *Pseudomonas* (1.59); *Trichoderma* (1.57), while the lowest content was produced by *Xanthomonas* (1.42); microbial contaminants (1.45) and mixed microbial isolates (1.49 mg/g fresh weight) compared with control (healthy and PVY-infected plantlets, 1.25 and 1.35 respectively).

The highest peroxidase activity was induced by *Fusarium* sp. 185.1, *Bacillus* sp. 180.5, and PVY-infected 175.2 while the lowest was induced by contaminant microbial 152.2 compared with healrth control 65.5 µg/g fresh weight.

Potato plantlets infected with PVY and treated with individual microbial isolates produced increasing on polyphenoloxidase activity (**Table 5**). *Fusarium* sp. and *Bacillus* sp. were found to be able to induce the highest activity of PPO in plantlets 221.3 and 215.7 unit/g fresh weight. While the contaminant microbial induced the lowest activity 199.2 unit/g.fw compared with healthy control 115.2 unit/g.fw.

On the other hand, the PVY-infected plantlets induced the highest PPO activity 265.3 unit/g.fw then the individual microbial and contaminants inducers.

Chlorophyll content:

PVY infection due to reduction in Chl a, Chl b and carotenoids contents related to healthy potato plantlets; it was 1.45, 1.81 and 1.25, respectively.

Generally, potato plantlets treated with filtrates of microbial individual and contaminants due to increasing in Chl a, Chl b and carotenoids contents compared with infected ones (**Table 5**).

Microbial contaminants due to increasing in salicylic acid content as well as PVY- infected potato plantlets related to healthy ones. It was 150.75 (*Pseudomonas*); 114.25 (*Xanthomonas*); 239.15 (*Bacillus*); 215.5 (*Trichoderma*); 220.15

(*Aspergillus*); 225.75 (*Fusarium*); 195.20 (mixed microbial isolates); 175.25 (microbial contaminants); 200.25 (PVY-infected) and 75.25 µg/g fresh weight (healthy control) (**Table 5**).

Table 5. Effect of microbial contaminants culture filtrates on some phytochemical contents of PVY-infected potato plantlets *in vitro*.

	Protein content (µg/g fresh weight)	Peroxidase specific activity (µg/g fresh weight)	Polyphenoloxidase specific activity (µg/g fresh weight)	Total SA (µg/g fresh weight)	Photopigments (µg/g fresh weight)		
					Chl a	Chl b	carotenoids
Health control	1.25	65.5	115.2	75.25	2.15	1.49	1.75
PVY-infected control	1.35	175.2	195.3	200.25	1.45	1.21	1.25
Microbial contaminants	1.45	155.2	195.2	175.15	2.25	1.68	1.72
Pseudomonas	1.59	173.5	185.7	150.75	2.27	1.71	1.92
Xanthomonas	1.42	152.5	136.2	114.20	2.51	1.91	2.15
Bacillus	1.75	180.5	215.7	239.15	2.75	2.05	2.12
Trichoderma	1.57	135.75	175.2	215.50	2.72	2.01	2.15
Aspergillus	1.80	124.2	175.4	220.15	2.41	2.31	1.95
Fusarium	1.70	185.1	221.5	225.75	2.50	2.40	1.85
Mixed microbial isolates	1.47	151.2	185.2	185.50	1.50	1.45	1.75

Protein related microbial:

Qualitative of protein related microbial contaminants were determined by SDS-PAGE. Bands with the same mobility were treated as identical patterns. Weak bands with negligible intensity and smear bands were both excluded from final analysis. **Figure (1)** demonstrates the SDS-PAGE profile obtained with seven biotic inducers. The number of scored new bands varied from 2 to 10. The total number of protein related microbial contaminants

and individual were 25 new bands consist of 3, 3, 2, 4, 3, 2 and 3 for microbial contaminants, *Ps.*, *Xanth.*, *B.*, *Trich.*, *Asp.* and *F.*, respectively as well as 4 bands for infected potato plantlets compared with healthy ones. The molecular weight ranged from 13.00 to 132.5 KDa.

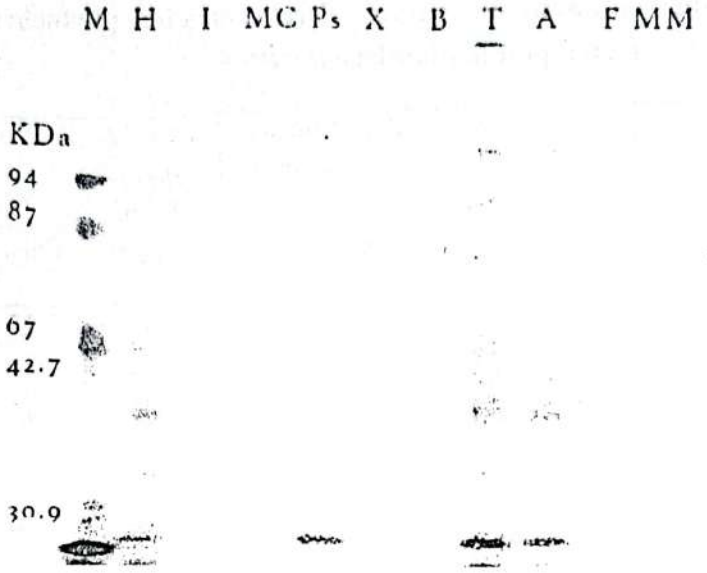

Fig. (1): SDS-PAGE of new proteins induced in potato plantlets infected with PVY and treated with microbial contaminants. M) Protein marker; H) Healthy; 1) PVY-infected; MC) Microbial contaminants; Ps) *Pseudomonas*; X) *Xanthomonas*; B) *Bacillus*; T) *Trichoderma*; A) *Aspergillus*; F) *Fusarium* and MM) Mixed microbial isolates.

Discussion

Minimum contamination in potato sprouts was obtained by sodium hypochloride and ethanol as surface sterilizing agents per culture can reduce or eradicate external contaminants. This result was suggested by **Mona Hussein (2007).**

Contaminants of micropropagated potato shoots were isolated using suitable specific media. Gram negative bacteria were mostly often found associated with plants and soil. The bacterial contaminants were assigned to major genera (*Enterobacter*, *Klebsiella*, *Corynebacterium* *serratia*,

Pseudomonas, *Bacillus*, *Xanthomonas*) based on the characteristics. The occurrence rate of bacterial isolates was 3٢ er than that of fungal isolates. Meanwhile, the most predominant fungal contaminants were *Fusarium* sp. followed by *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Rhizopus* sp., *Mucor* sp. and *Candida* sp. These results are in harmony with the results obtained by **Leifert and Cassells (2001)**, who found that a wide range of microorganisms (filamentous fungi, yeasts, bacteria, viruses and viroids) and micro-arthropods (mites and thrips) have been identified as contaminants in plant tissue cultures. Contaminants may be introduced with the explant during the manipulations in the laboratory or by micro arthropod vectors. Contaminants may express themselves immediately or can remain latent for long periods of time. This often makes it difficult to identify the source of contamination.

It worthy to mention that the isolates of *Pseudomonas* and *Bacillus* genera were the highest frequency, whereas, the lowest frequency of bacteria isolates was observed with *Enterobacter*, *Klebsiella* and *Corynebacterium* genera. Earlier investigators reported that many bacterial contaminants were isolated during tissue culture procedures from several plant cultures such as gram positive and gram negative bacteria were *Staphylococcus xylosus*, *S. aureus*, *S. cohnii*, *Bacillus* sp., *Corynebacterium* sp., *Micrococcus* sp., *Pseudomonas vesicularis*, *Serratia* sp., *Cellulomonas* sp., *Clavibacter* sp., *Curtobacterium*, *Microbacterium* sp., *Acinetobacter* sp., *Wautersia* (*Ralstonia*) and *Stenotrophomonas* sp. **(Van Den Houwe and Swennen, 2000; Bhattacharya, 2002; Lata *et al.*, 2006; Mona Hussein, 2007).**

On the other hand, many fungal contaminants were isolated during tissue culture procedures from several plant cultures. These contaminant fungal isolates were *Aspergillus* sp., *A. niger*, *A. fumigatus*, *Alternaria* sp., *Al. tenius*, *Candida* sp., *Fusarium* sp., *F. culmorum*, *Geotrichum* sp., *Helminthosporium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. **(Murugesh *et al.*, 1999; Ramirez-Villalobos *et al.*, 2000; Acosta *et al.*, 2002;**

Odutaya *et al.*, 2004; Wu, 2005; Mona Hussein, 2007).

As regard to the effect of bacterial cultures filtrate on shoots and roots as well as growth value showed that the highest shoots and roots length as well as growth value were obtaining from applications of bacterial filtrate. As well, it is worthy to mention that the filtrate of *Pseudomonas* culture showed higher promotion effect on strawberry growth value in comparison with addition of the filtrate of *Bacillus* culture. In this concern, several research as demonstrated that *Xanthomonas* and *Pseudomonas* produced IAA in their cultures (**William *et al.*, 1987**). Also, **Lata *et al.* (2006)** reported that *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Wautersia (Ralstonia)* and *Stenotrophomonas* produced IAA in their cultures. These findings suggested that, microbial auxins may be promoting the growth parameters under tissue culture conditions.

Regarding the effect of fungal culture filtrate application to the potato culture medium showed that the fungal filtrate enhanced the all potato plantlets growth parameters compared with control treatment (without fungal filtrate). The enhancement was increased with the increasing of fungal filtrate application. Moreover, it is worthy to mention that the filtrate of *Fusari* 34 culture showed higher promotion effect on potato plantlet lengths and growth value as compared to the filtrate of *Aspergillus* sp. culture.

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