RESEARCH | Egyptian Journal of Agricultural Research | PLANT PATHOLOGY

Management of probiotic bacterial contamination and plant stimulants of PVY-infected potato plantlets on potato tissue culture in vitro

Noha K. Eldougdoug[1]; Tarek E. Abdelbaset[2]; Amira M. Ismaeil[2]; Mohamed S. Attia[3]; Amer M. Abdalazez[1]; and Rehab A. Dawood[4]*

Address:

[1]*Botany and Microbiology Department, Faculty of Science, Banha University, Egypt*

[2]*Virus and Phytoplasma Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt*

[3]*Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt*

[4]*Department of Biology, Faculty of Science, Jazan University, Box 114, Jazan 45142, Saudi Arabia*

*Corresponding author: ***Tarek E. Abdelbaset.*** email: drtarekelsayed71@gmail.com

Received: 13-02-2025**; Accepted:** 14-02-2025**; Published:** 0-00-2025 **DOI:** 10.21608/EJAR.2025.315871.1578

ABSTRACT

Potato virus Y (PVY) is causing a serious loss in yield and quality of potatoes. The present study shows the effect of antiviral Ribavirin and antibacterial Bacteriocin on regeneration response and production of PVY-free plants under *in vitro* conditions. PVY-infected and healthy potato meristem tips were transplanted in Murashige, & Skoog, (MS) medium supplemented with 0.1 mg L^{-1} GA_3, 0.1 mg L^{-1} NAA, and 500 mg L^{-1} malt extract, ribavirin or/and Bacteriocin and the acquired resistant and reduction of virus in plantlets were determined. DAS-ELISA and RT-PCR were used to index the mother plant and *in vitro*-regenerated plantlets for virus indexing. *In vitro,* regenerated plantlets tested negative in both ELISA and RT-PCR were only considered as virus-free. The bacterial and fungal contaminants were isolated and identified based on morphological and cultural characteristics of major genera that included, *Pseudomonas, Bacillus, Serratia, Xanthomonas, Aspergillus, Penicillium, Geotrichum, Trichoderma,* and *Alternaria.* MS medium treated with ribavirin or Bacteriocin suppressed contaminated bacteria and fungus. Consequently, plant height, leaf size, number of branches, and root number all significantly increased, in addition to plantlet growth. In addition, PVY-free potato plantlets were produced, as validated by the DAS-ELISA analysis. For more, When compared to untreated plantlets, bacteriocin and/or generate inducers enhanced protein content and oxidative enzyme activities (PO, PPO), salicylic acid, Chl a, Chl b, and carotenoids in potato healthy plantlets and PVY-infected plantlets. The current study suggested that the use of ribavirin and/or Bacteriocin improves the quality of potato plantlets grown in tissue culture.

Keywords: PVY; Probiotic bacteria; Ribavarin, Bcteriocin.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important vegetable crop grown in 80% of the countries in the world and is consumed in large quantities by millions of people. It is relatively a cheap source of calories and contains important health-promoting phytonutrients (Ezekiel *et al.,* 2013). It has been observed that the high incidence of viral diseases is responsible for the low yield of potatoes in major potato-producing countries of the world. In Egypt, potato is an important crop with 5.1 million tons produced in (Central Agency for Public Mobilization and Statistics. 2018). According to the Food and Agriculture Organization (FAO) statistical database, it makes the country the sixth-largest potato exporter in the world. Many countries rely on Egypt's exports of potatoes, which are approximately 734 thousand tons (FAO 2021). Viral diseases are considered the major constraint in many countries, resulting in production losses (Tamisier *et al.,* 2022). Potato virus Y (PVY) is the most economically important virus affecting potato production worldwide (Sergio *et al.,* 1996). *Potato virus Y* (PVY) was the most prevalent virus found by Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (Kreuze *et al.,* 2020). Providing virus-free planting material is one of the most challenging aspects of potato cultivation. The use of antiviral chemicals in culture medium during regeneration of plantlets has been proven to be successful in producing virus-free plants in many plant species (Ram *et al.,* 2005 and Simpkins *et al.,* 1981).

Tissue culture media is a good source of nutrient for plant and microbial growth. Ribavirin prevents viral replication in its early stages. All ribo- and deoxyribonucleosides, principally thymidine, reversed the inhibition to varying degrees (Tsai *et al.,* 2006). Nucleosides compete with ribavirin in tobacco leaves for

phosphorylation to monophosphate by a nucleoside phosphotransferase (Benčić *et al.,* 2023). On the other hand, triphosphate is ribavirin's primary and last phosphorylation product. Ribavirin triphosphate is thought to be an antiviral that works by preventing viral RNA capping (Ferron *et al.,* 2012). This form of action can be used to suppress PVY, whose RNA is most likely covalently coupled to a protein at its 5'-terminus (Lerch, 1987). One of the most important issues in tissue culture is microbial contamination (Mahmood and Ali, 2017). A wide range of microorganisms (fungi, yeasts, bacteria, Actinomycetes and viruses) have been identified as contaminants in plant tissue cultures (Leifert and Cassells, 2001). Microbes adversely compete with plant tissue culture for nutrients**.** As well as viral infection due to poor growth of the plantlets and death. A-Ling Zhang *et al.,* (2024) indicated that chemotherapy-based methods with ribavirin treatments in viro are effective in eradicating kiwifruit viruses in mixed infections. Bacteriocins are active antimicrobial proteins produced by bacteria against gram positive and gram negative bacterial pathogens (Khattab *et al.,* 2021) and recently used against pathogenic fungi including Candida sp. and *Aspergillus* sp. Bacteriocin produced by Lactobacillus strains can be used as a probiotic bacterium to inhibit other bacterial pathogens. This bacteriocin inhibited some bacterial pathogens in culture collection including *Enterococcus faecalis* ,*Staphylococcus aureus* , *Streptococcus pyogenes* , *Listeria monocytogenes* , *Bacillus cereus* , *Pseudomonas aeruginosa* , and *Burkholderia cepacia* (Mathur *et al.,* 2017; Chikindas *et al.,* 2018; Macaluso *et al.,* 2016).Recently, microorganisms have been used as plant growth stimulants, and have also been used to improve plant resistance against biotic and abiotic stresses(Attia *et al*., 2022; Khattab *et al.,* 2022; Attia *et al*., 2023a; Attia *et al.,* 2023b). The use of *Trichoderma* as a plant growth enhancer, as well as to stimulate plant physiological responses against plant diseases, is considered one of the most important modern weapons in biological resistance against plant diseases (Abdelaziz, *et al.,* 2023c; Hashem, *et al.,* 2023a). The present work aims to screen different microbial contaminants in plant tissue culture and study the effect of biotic inducers to induce systemic resistance in potato plantlets against Potato virus Y in vitro and (bacterial and fungal) contaminants.

MATERIALS AND METHODS

1. Virus source and samples collection:

One hundred potato tuber sprouts infected with PVY, and twenty healthy local fracture nodes (1-2) (*Solanum tuberosum* cv. Spounta) were produced in the Virology Greenhouse at the Faculty of Agriculture, Ain Shams University. DAS-ELISA was used to confirm the infection using polyclonal antibodies (Clark and Adams, 1977). The sprouts underwent a five-minute surface sterilization using 3% sodium hypochlorite, followed by three rinses in sterilized distilled water. Under a stereomicroscope (CETI WF 10X), meristem tips approximately 3.0 mm in length were excised and cultured on MS medium in a jar (250 ml) (Murashige and Skoog, 1962) and then incubated at 16°C. Daylight at 18ºC was maintained for 30 days in the shoot differentiation medium. Gelrite (1,2,4-triazole-3-carboxamide) from Sigma-Aldrich Co. LLC, 2 g in 1,000 ml of distilled water, was incorporated into the plant tissue culture medium (Hanafy *et al.,* 2016). It was heated while stirring frequently, simmered for one minute to fully dissolve, and then autoclaved for 15 minutes at 121°C and 15 psi.

2. The Probiotic bacteria strain (*Lactobacillus acidophilus* CL1285) was obtained from Culture Collection (MIRCEN), Ain Shams Univ., Cairo, Egypt.

2.1. Production and Purification of bacteriocin:

Lb. acidophilus ($5x10^6$cfu / ml^1) was inoculated on MRS broth medium (De Man *et al.,* 1960) for 24 hrs at 30ºC. Cell free supernatants (CFS) were collected by centrifuging the culture broth at (6,000 rpm for 20 minutes at 4ºC).

Experimental design:

Twenty jars per treatment, each containing five plantlets, were used for Ribavirin, and another twenty jars for Bacteriocin. These were cultured in a semi-solid MS medium consisting of basic MS salts, 0.04 mg/L Kinetin, 1 mg/L IAA, 3% sucrose, and Gelrite (2.0 g/L), supplemented with 20.0 mg/L of antiviral Ribavirin and 5 µL/mL Bacteriocin to study their effects on PVY elimination and bacterial and fungal contaminants. The growing meristem was subcultured to a fresh medium every four weeks. All cultures were incubated at 26 ± 2°C with 16 hours of light and 8 hours of dark for three weeks. The cultures were examined weekly, and the contamination rate was calculated for bacterial and fungal contaminants.

2.2. Isolation and Identification of microbial contaminants:

Bacterial contamination during micropropagation in potato cultures was counted on a nutrient agar plate medium and incubated at 37°C for two days (Jay *et al.,* 2008). Additionally, fungal contaminants were counted using Acidified Potato Dextrose Agar medium, cultured at 26°C for five days. The bacterial contaminants were detected using their morphological features and cultural traits. The VITEK2 system then confirmed their primary genera identity. A single colony of a pure culture was transferred using a disposable bacterial needle, and the microbe was suspended in 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a

clear polystyrene test tube measuring 12 by 75 mm. The turbidity was changed according to (Table 1) and assessed using a turbidity meter called the Densi Chek.

Table 1. Suspension turbidities used for card inoculation

McFarland Turbidity Range	Product
0.50-0.63	GN
0.50-0.63	GP

3.PVY detection and concentration: The percentages of survival and PVY-free plantlets were recorded. DAS-ELISA assayed PVY detection and concentration according to (Clark and Adams, 1977).

3.1. Numbers, length of shoots and roots, as well as growth value, were calculated in each treatment throughout the subculture after 8 weeks according to (Ziv, 1992).

3.2. Total soluble proteins were assessed by the method of (Lowry *et al.*, 1951) using casein as a standard with a UV spectrophotometer (UNICO 2000) set to 750 nm in wavelength to measure the total protein content of leaves.

3.3. Enzyme activities: One gram of plantlet tissue was ground with 3 mL of potassium phosphate (50 mM, pH 7.0). The homogenates were centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant (crude enzyme protein) was collected and divided into 1.5 mL portions.

3.3.1. Peroxidase activity (PO) was determined in 200 µL of the crude enzyme with 3.5 mL phosphate buffer (pH 7.0, 0.1 M) and mixed with 100 µL guaiacol (0.251). The optical density was recorded every minute for 5 minutes at 470 nm (Malik, 1980).

3.3.2. Polyphenol oxidase activity (PPO) was determined 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 minute for 5 minutes at 420 nm, according to (Coseteng and Lee, 1987).

3.3.3. Chlorophyll and carotenoids were extracted and determined according to (Abd Alhakim *et al.,* 2022).

3.3.4. Salicylic acid was extracted and quantified from potato plantlets by fluorescence HPLC according to (Loake and Grant, 2007). Expressed proteins were detected using SDS-PAGE based on the method by (Studier, 1973).

Statistical analysis: One-way ANOVA and the Holm-Sidak test (SigmaPlot 12.0) were used to analyze the data at a 0.05 level of probability. The recorded values from the biochemical analysis are the means of three replicates.

RESULTS

One hundred potato tubers of the cultivar Spunta were tested for virus infection (PVY, PVX, and PLRV) using a DAS-ELISA assay, which is used to examine PVY-free plants as well as micropropagated plantlets. It was found that the highest percentage of PVY infection was 12.5%, followed by PVX infection at 0.8% and PLRV infection at 2.5%. Some tubers showed mixed viral infections: PVX + PVY at 1.5%, PVY + PLRV at 3.5%, PVX + PLRV at 1.6%, and a mixed infection by viruses PVX + PVY + PLRV at 1.5%. Additionally, 75.32% of the potato tubers cv. Spunta was healthy according to the DAS-ELISA results.

Effect of ribavirin on PVY and production of potato cultures and sanitized plantlets:

Meristem tips (2 mm) from infected plantlets were cultivated on MS medium and incubated at 21°C for 3 weeks. It was found that the survival percentage in control plantlets (medium free from Ribavirin and Bacteriocin) was 100%; plantlets in contaminated media had 65%; plantlets in media treated with Ribavirin had 95%; plantlets in media treated with Bacteriocin had 98%; and plantlets in media treated with Ribavirin + Bacteriocin had 98%. In contrast, the survival percentage for healthy potato plantlets was 5%, 7%, 75%, 63%, and 78%, respectively. Infected potato plantlets showed survival percentages of 95%, 58%, 20%, 35%, and 20%, respectively. While PVY elimination was 0.5%, 10.8%, 78.9%, 64.3%, and 79.6% respectively, these results were confirmed by the DAS-ELISA assay (Table 2).

Table 2. Effect of MS medium supplemented with Ribavirin and bacteriocin on PVY infected plantlets micro propagated

Treatment PVY infected Plantlets	No. of Plantlets	No. of survival		%PVY elimination
		Healthy	Infected	
Control media*.	100	5	95	5
Contaminated media	65	7	58	10.8
Media Treated with Ribavirin	95	75	20	78.9
Media Treated with Bacteriocin	98	63	35	64.3
Media Treated with Ribavirin + Bacteriocin	98	78	20	79.6

*Media free from Ribavirin and Bacteriocin.

Identification of microbial contaminants:

Identification of bacterial isolates: The bacterial contaminants were identified by morphological criteria and verified with the VITIK2 procedure. The major genera identified, along with their occurrence frequency, were 35.56% (*Pseudomonas* spp), 31.75% (*Bacillus* spp), 15.75% (*Xanthomonas* spp), and 16.94% (*Serratia* spp) (Table 3). The biochemical characteristics of the isolated bacteria were confirmed with an excellent probability of 99% after full biochemical identification by the VITIK2 system.

Table 3. Morphological, cultural characteristics and frequency of contaminant bacterial isolates of potato micropropagated plantlets.

Bacteria	Bacterial Characteristics					
	Cell morphology	Mot.	G.S	Endospore formation	Pigment production	Freq. (%)
***Pseudomonas* sp.**	Short rod	+	G-	No	Water soluble greenish yellow	35.56
***Bacillus* sp.**	Long rod	+	G+	Intermediate spore	Creamy	31.75
***Xanthomonas* sp.**	Short rod	+	G-	No	Pale yellow	15.75
***Serratia* sp.**	Short rod	+	G-	No	Dark red inside cell	16.94

Mot. = Motalily ,**G.S** = Gram stain **Freq.** = Frequency

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

Identification of fungal contaminants: Fungal contaminants were identified based on morphological, spore formation, and cultural characteristics. The major genera, with their occurrence frequency, included: *Aspergillus* spp. (36.00), *Penicillium* spp. (25.75), *Trichoderma* spp. (15.75), *Geotrichum* spp. (15.10), and *Alternaria* spp. (7.50) as shown in (Table 4 and Fig. 1).

Table 4. Morphological characteristics and frequency of contaminant fungi isolates of micropropagated plantlets.

Fungal isolates	Colony shape	Characteristics of the genus	Frequency (%)
***Aspergillus* sp.**	Fuzzy black color	Mycelium septated, 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.	36.00
***Penicillium* sp.**	Fuzzy greenish color	Mycelium septated, 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 a yellow color	25.75
***Trichoderma* sp.**	Fuzzy greenish color	Mycelium septate, septate conidiophore sterile at the tip, conidia ellipsoidal tuberculate, transverse septa conidia usually in chains and sometimes present bears and yellow-green pigment.	15.75
***Geotrichum* sp**	Platen	Yeast-like .unregulated cylindrical cells, sometimes produce pseudomycelium , budding oval cells	15.10
***Alternaria* sp.**	Fuzzy black color	Mycelium septated, short conidiophores carry chains of conidia spores, conidia are large and multicellular, longitudinal; transverse septa conidia usually in chains and sometimes present single and dark yellowish.	7.50

Effect of Adding a Biotic to MS Medium on Bacterial and Fungal Contaminants:

Antimicrobial ribavirin and bacteriocin were applied against bacterial contamination. The results showed that adding 20 mg/L of ribavirin and bacteriocin significantly reduced bacterial growth. The reduction rate was higher with the combination of bacteriocin and ribavirin (0.23, 0.40, 0.47, and 0.6), followed by bacteriocin alone (1.28, 1.31, 1.25, and 1.23 CFU/ml), and then ribavirin alone (3.90, 3.23, 3.25, and 2.35) against *Bacillus* sp., *Pseudomonas* sp., *Xanthomonas* sp., and *Serratia* sp., respectively (Fig. 1).

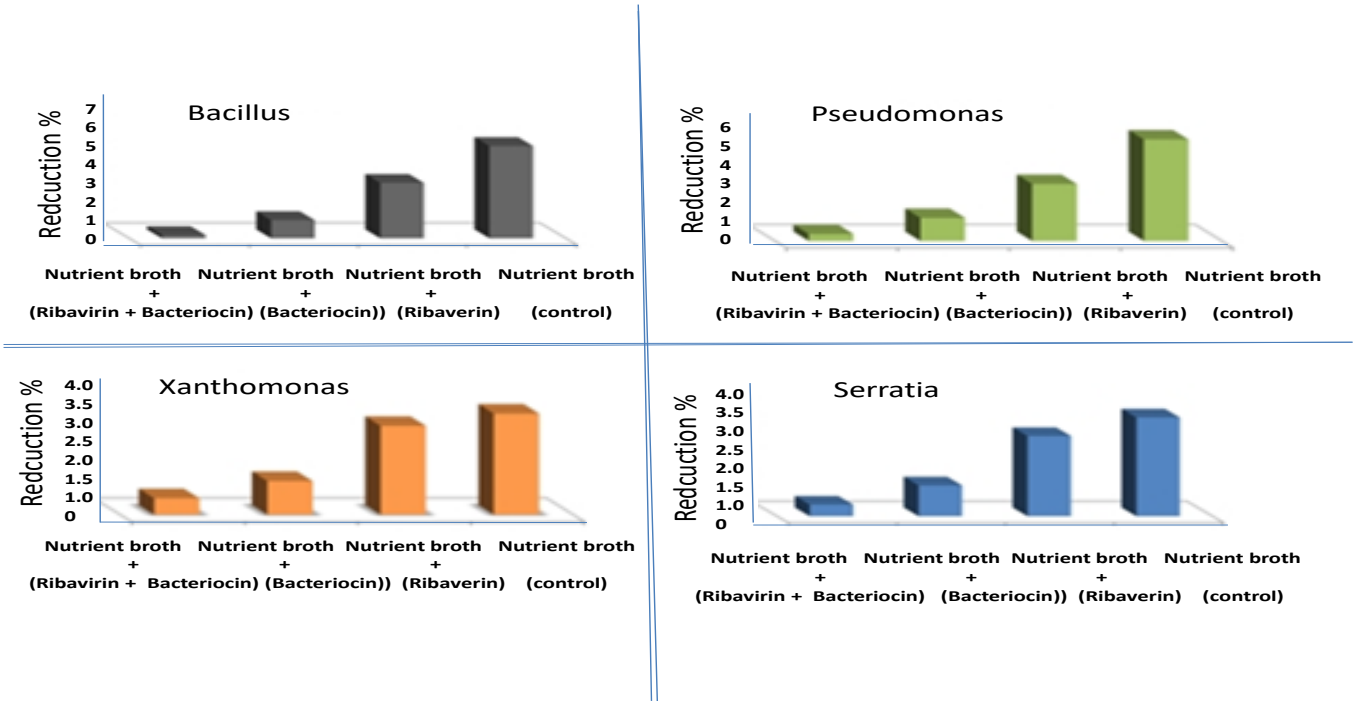

Fig. 1. Effect of adding a biotic to MS medium on bacterial and fungal contamination.

On the other hand, potato dextrose agar (PDA) is supplemented with ribavirin and bacteriocin to reduce contaminated fungal growth. The reduction rate was higher with both bacteriocin and ribavirin (0.75, 0.84, 0.65, 0.68, and 0.95), followed by bacteriocin alone (1.23, 1.25, 1.62, 1.75, and 1.84), while ribavirin alone showed rates of (2.5, 2.32, 3.05, 2.90, and 3.20) Log (CFU mL-1) for *Aspergillus* sp., *Alternaria* sp., *Trichoderma* sp., *Penicillium* sp., and *Geotrichum* sp., respectively (Fig. 2).

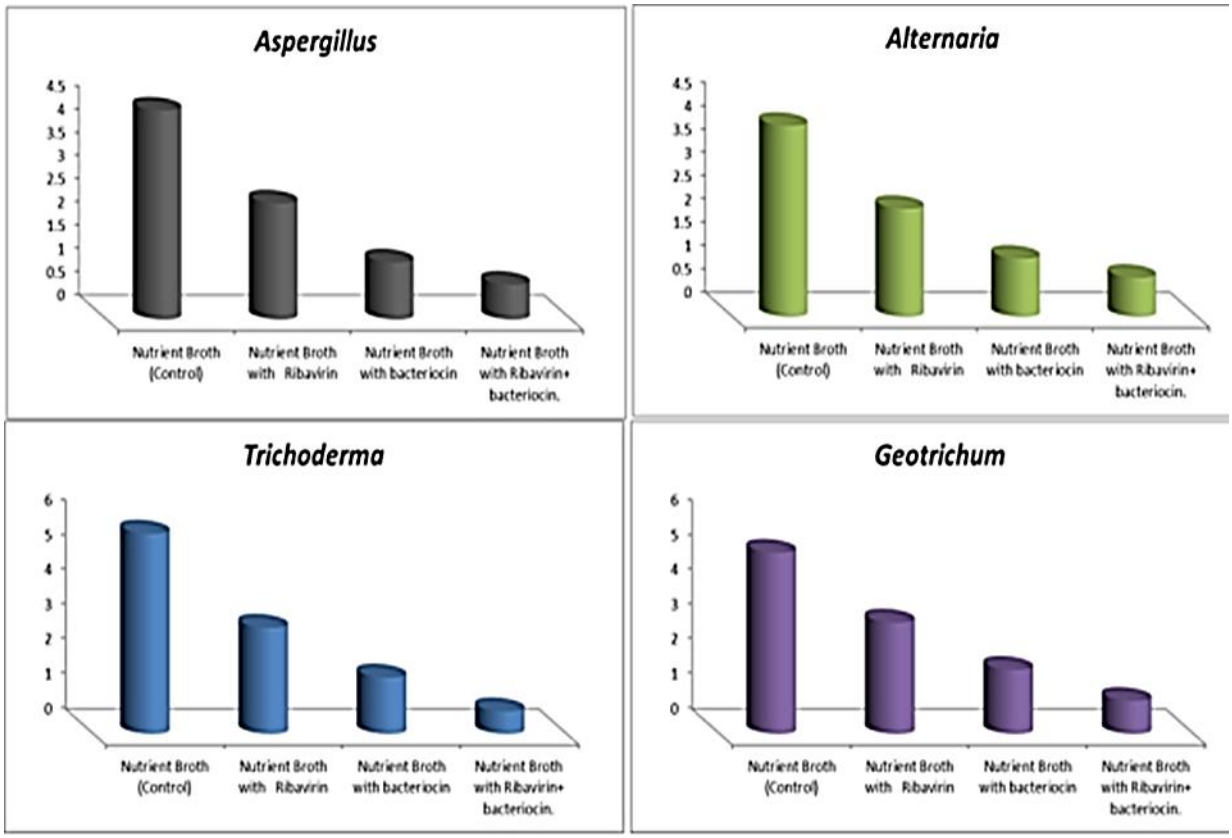

Fig. 2. Effect of Ribavirin and Bacteriocin on fungal Growth rate.

Induced acquired resistance (IAR) in micro propagated potato plantlets*:*

The indicators of IAR related to treatments are based on the reduction of PVY infectivity and the growth rate of potato plantlets, as well as phytochemical indicators, protein content, PO, PPO specific activities, total SA, and photo pigments.Virus infectivity: The PVY isolate was detected in potato sprouts using specific polyclonal antibodies by DAS-ELISA. MS medium supplemented with ribavirin and bacteriocin reduced PVY infection. The highest rate of reduction was found with ribavirin + bacteriocin (89.3%), followed by ribavirin alone (83.9%) and bacteriocin alone (77.8%), compared to MS without ribavirin and bacteriocin (5%). As well as, culture filtrates of microbial contaminants, 6.1% (Table 5). On the other hand, the virus concentration was reduced in relation to treated ribavirin and bacteriocin, and Ribavirin + bacteriocin with MS medium, showing 0.275, 0.215, and 0.185 OD using DAS-ELISA, respectively, compared with MS without ribavirin and bacteriocin at 0.425 OD. Additionally, culture filtrates of microbial contaminants showed 0.325 OD.

Table 5. Effect of Ribavirin, bacteriocin and culture filtrates of microbial contaminants on PVY infected potato plantlets.

Parameters Plantlet treatments	PVY infectivity in potato plantlets			
	No. of Plantlets	**No of PVY infected Plantlets**	**(%) Reduction infection**	***Optical density**
MS (control) *	100	95	5.0	0.425
Culture filtrates	65	58	10.8	0.325
MS treated with Bacteriocin	95	20	73.7	0.275
MS treated with Ribavirin	98	15	84.7	0.205
MS treated with Ribavirin +Bacteriocin	98	10	89.8	0.185
P value	P < 0.001	P < 0.001	P < 0.001	----
LSD	6.34	5.78	5.12	----

P < 0.001 indicated highly significant differences in infection rates between treatments.

LSD at 5% significance level demonstrated that the infection rate in MS (control) group was significantly higher than all treatments. While (Ribavirin and Bacteriocin) combination resulted in reduction of infection rate, significantly lower than all other treatments.

***Optical density= mean of ten replicates (five plantlet/jar) -Plantlet PVY infected (+ve) = 0.375 OD - Plantlet healthy (-ve) = 0.125 OD

Potato plantlets growth index:

Regarding the supplementation of Ribavirin and bacteriocin to the MS medium culture, there was an enhancement in shoot growth (length, number), leaf number, and root growth (length, number) compared to the control (healthy or PVY-infected potato plantlets) as shown in (Table 6). The rate of shoot growth significantly increased with the combination of ribavirin and bacteriocin, followed by each treatment individually, compared to both healthy and PVY-infected plantlets. It is worth mentioning that bacteriocin, culture filtrates of microbial contaminants, and (Ribavirin and bacteriocin) showed a higher promotion effect on potato plantlet growth compared to healthy and PVY-infected plants, as shown in (Table 6 and Fig. 3).

Table 6. Effect of Ribavirin, bacteriocin and culture filtrates of microbial contaminants on growth parameters (Shoots Length, Shoots Number, Leaves Number, Roots Length and Roots Number) of PVY infected potato plantlets.

Treatments	Shoots		Leaves number	Roots	
	length (cm)	**Number**		**length (cm)**	**Number**
Healthy plantlets.	5.35	3.0	5.0	1.75	5.25
PVY infected plantlets.	4.15	1.0	3.0	0.72	3.75
PVY infected plantlets treated Culture filtrates	4.25	1.0	4.0	0.35	3.27
PVY infected plantlets treated with ribavirin	5.10	2.0	4.0	1.25	4.0
PVY infected plantlets treated with bacteriocin	7.75	2.0	4.0	1.82	5.75
PVY infected Plantlets treated with ribavirin +bacteriocin	8.50	3.0	4.0	2.10	6.0
P-value*	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
LSD (.05)	1.23	1.11	0.78	0.45	0.92

***P-value** (**P < 0.001)** indicated highly significant differences among treatment groups

** Least Significant Difference (LSD) analysis at 5% level revealed significant differences between treatment means for all measured variables.

Fig. (3). Photo showing PVY-infected potato plantlets (V) growth on MS medium in comparison with healthy (H) and PVY-infected treated with Ribavirin (V+R), bacteriocin(V+B) , culture microbial filtrates (V+F) and PVY-infected treated with Ribavirin , bacteriocin, culture microbial filtrates (V+R+B) .

Phytochemical indicators:

Phytochemical indicators measured the induced acquired resistance in potato plantlets. High significance in protein content was observed with ribavirin, bacteriocin, bacteriocin + ribavirin, and culture filtrates of microbial contaminants, while low significance was noted with ribavirin alone compared to healthy and PVY-infected plantlets. On the other hand, virus infection significantly decreased protein content compared to healthy plantlets. The highest peroxidase activity was induced by bacteriocin and ribavirin at 185.5 µg/g and by bacteriocin alone at 165.2 µg/g, as well as by culture filtrates of microbial contaminants. The lowest peroxidase activity was induced by ribavirin at 125.2 µg/g, compared with healthy plantlets at 75.8 µg/g and PVT-infected plantlets at 105.2 µg/g fresh weight. A similar trend was observed for potato plantlets infected with PVY and treated with ribavirin and/or bacteriocin, due to a significant increase in polyphenol oxidase activity (Fig. 4).

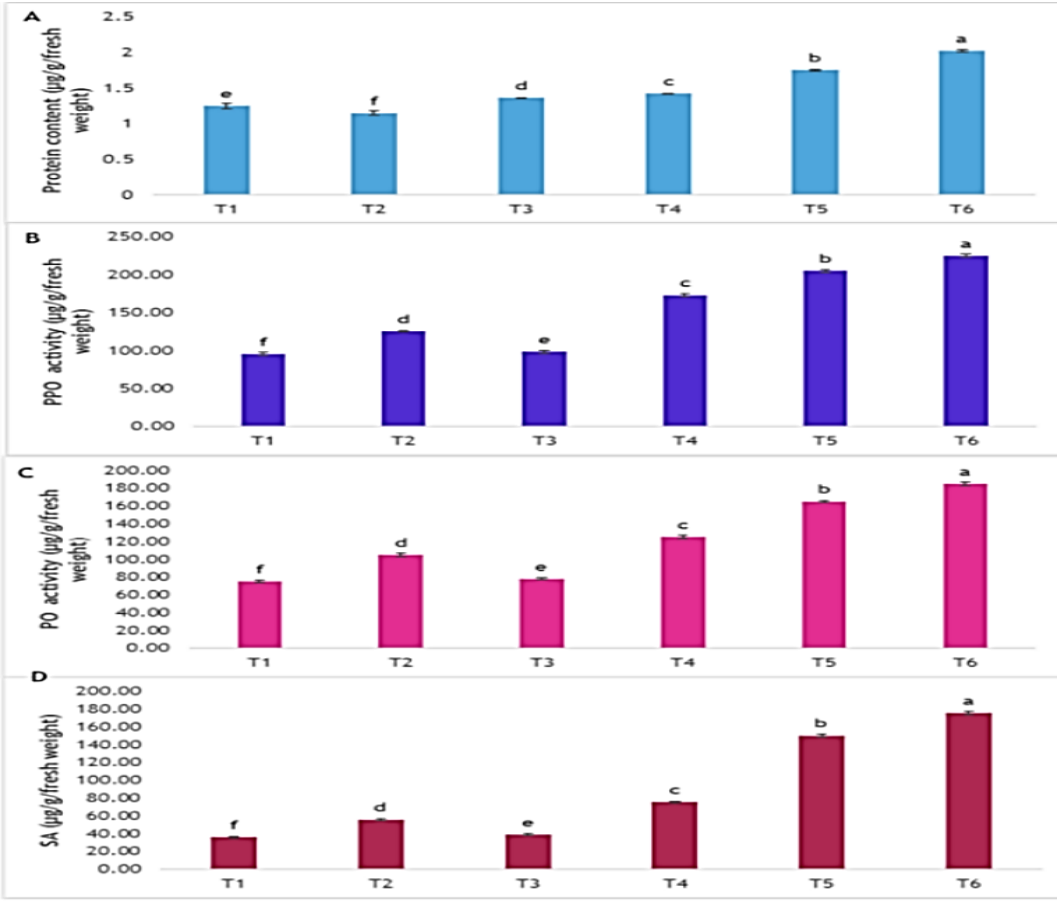

Fig. 4. Effect of Ribavirin, Bacteriocin, and culture filtrates of microbial contaminants on photosynthetic pigments: (A) protein content. (B) PPO activity (C) PO activity and (D) SA contents of PVY infected potato plantlets. T1 = Helthy control; T2 = Infected control; T3 = infected plantlets treated culture filtrates; T4 = infected plantlets treated with ribavirin; T5 = infected plantlets treated with bacteriocin; T6 = infected plantlets treated with ribavirin + bacteriocin. Calculated as average from 100 potato plantlets (10 jars). Letters reversed to significant in static analysis.

PVY infection leads to a reduction in Chl a, Chl b, and carotenoid contents in infected potato plantlets compared to healthy ones; these levels were (1.75, 1.25, 1.15) µg/g fresh weight and (3.75, 2.75, 1.72) µg/g fresh weight, respectively. Generally, potato plantlets grown on MS medium supplemented with bacteriocin and ribavirin show increased Chl a, Chl b, and carotenoid contents compared to those grown on MS-free medium when infected.

A biotic inducer results in an increase in salicylic acid content in PVY-infected potato plantlets compared to healthy ones. It was 75.45 (ribavirin), 150.25 (bacteriocin), 175.75 (bacteriocin), 55.75 (infected plantlets), and 35.75 (healthy plantlets) (Fig. 5).

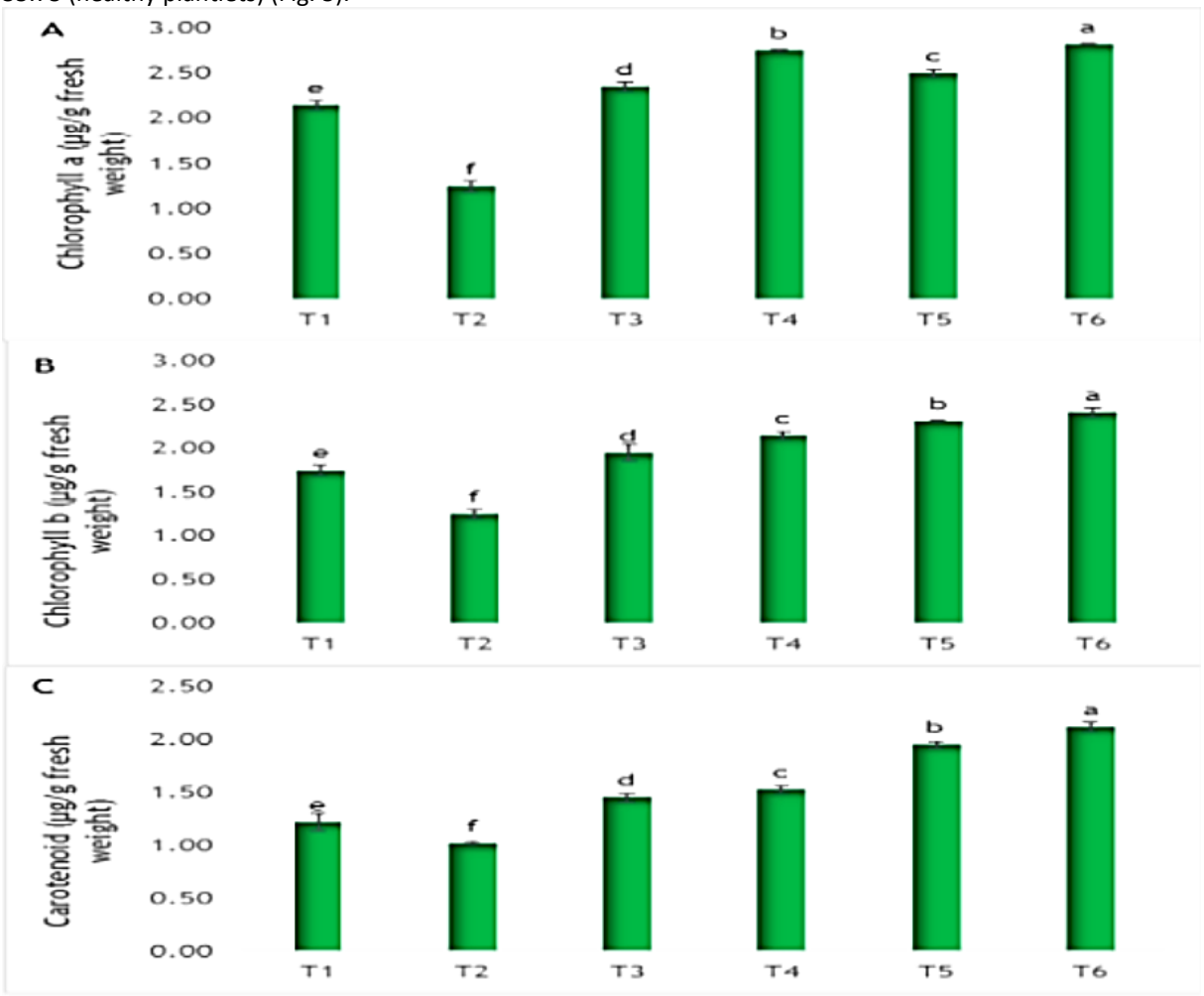

Fig. 5. Effect of Ribavirin, Bacteriocin, and culture filtrates of microbial contaminants on photosynthetic pigments: (A) chlrorphyll a. (B) chlorophyll b and c carotenoids contents of PVY infected potato plantlets. T1 = Helthy control; T2 = Infected control; T3 = infected plantlets treated culture filtrates; T4 = infected plantlets treated with ribavirin; T5 = infected plantlets treated with bacteriocin; T6 = infected plantlets treated with ribavirin + bacteriocin. Calculated as average from 100 potato plantlets (10 jars). Letters reversed to significant in statically analysis.

Expressed protein:

Expressed qualitative and quantitative protein-related abiotic inducers were determined by SDS-PAGE. The absence, presence, weak bands with negligible intensity, and smear bands were excluded from the final analysis. The SDS-PAGE profile demonstrated newly expressed protein bands with MW 95, 75, 66, and 38 KDa related to abiotic inducers: 95, 75, and 66 for ribavirin and 38 KDa for bacteriocin. The total number of proteins related to abiotic inducers individually was 25 new bands consisting of 3 for ribavirin and 6 of the polypeptide pattern for each of ribavirin + bacteriocin compared with healthy ones (Table 7 and Fig. 6). The polymorphism among abiotic stress showed specific bands (polymorphic) at 55%, common bands in all treatments (monomorphic) at 33.3%, and unique bands for ribavirin (genetic marker, 78 KD) at 11.1% (Table 9). The number and density of expressed proteins differed between abiotic stress conditions, with 6, 5, 8, and 7 bands for healthy, infected, and ribavirin-treated samples, respectively.

Table 7. Polymorphism expressed qualitative and quantitative of polypeptide patterns related a biotic inducers by SDS-PAGE.

MW KD	Healthy MS	Infected MS	Infected MS+ Ribavirin	InfectedMS+ bacteriocin.	Infected MS (Ribavirin+ bacteriocin.	Polymorphism
95	+	-	+	-	-	Polymorphic
82	-	+	++	+	+	Polymorphic
78	-	-	+	-	-	Unique
72	+	-	+	+	+	Polymorphic
66	+	+	+	+++	+++	Polymorphic
58	++	+	++	++++	++++	Monomorphic
45	++	+	+	+++	+++	Monomorphic
38	-	-	-	+++	++++	Polymorphic
29	++++	++++	++++	++++	++++	Monomorphic
Total	6	5	8	7	7	

MW KD= Molecular Wight Kilo Dalton
Unique = genetic marker
Polymorphic= specific of polypeptide pattern
Monomorphic= Common polypeptide pattern

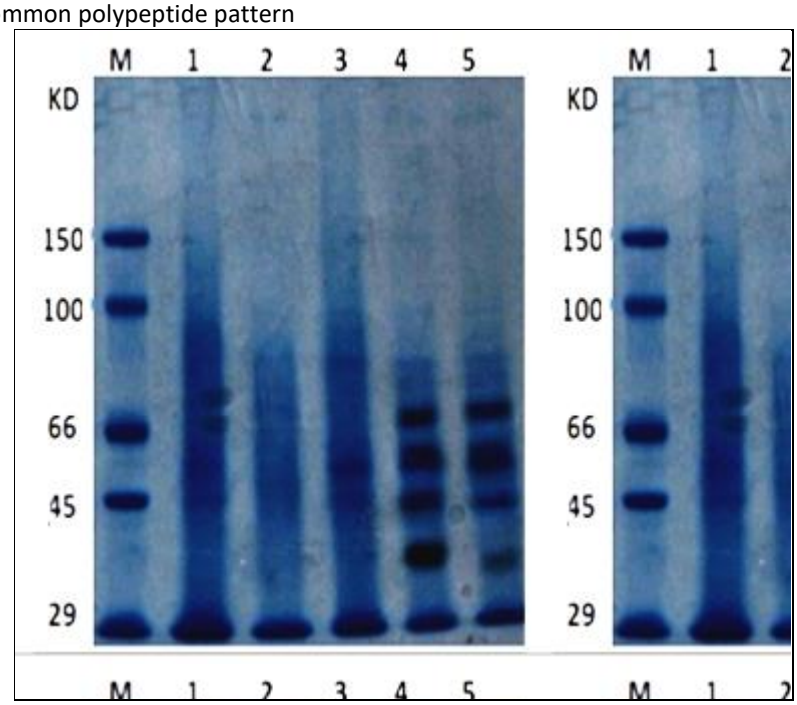

Fig. 6. Electrogram showing SDS-PAGE of expressed proteins related a biotic inducers (Ribavirin and Bacteriocin) induced in potato plantlets infected with PVY and healthy ones. (M) protein marker, (1) untreated healthy plantlets, (2) untreated PVY infected plantlets, (3) PVY infected plantlets on MS medium supplemented with Ribavirin, (4) PVY infected plantlets on MS medium supplemented Bcteriocin, (5) PVY infected plantlets on MS medium supplemented Ribavirin + Bcteriocin.

DISCUSSION

The main objectives of this study were the elimination of PVY and the improvement of growth in PVY-infected potato plantlet cultures during micropropagation using ribavirin as an antiviral and gelrite as a biotic inducer. Additionally, it aimed to decrease bacterial and fungal infections using the antibacterial activities of bacteriocin. Antiviral chemicals may have a selective inhibitory effect on viral nucleic acid synthesis (Fraser and Gerwitz, 1984). They block virus replication, preventing the virus present in old tissue from moving to new outgrowths, resulting in virus-free or low-virus plants (Simpkins *et al*., 1981). Ribavirin, as documented in the literature, is effective against RNA viruses by inhibiting the 5' capping of viral RNAs (Dawson and Lozoya-Saldana, 1984). The guanosine analog ribavirin also interferes with the guanosine 5’-phosphate (GMP) biosynthetic pathway. Ribavirin prevents the synthesis of new virus particles by inhibiting viral RNA synthesis, and existing virus particles are degraded during their development. The results of the present study align with earlier findings

that ribavirin is a potent inhibitor of plant viruses. Antiviral chemicals that are analogs of nucleic acid bases or nucleosides are very effective in blocking viral nucleic acid synthesis (Fraser and Gerwitz, 1984). 2-Thiouracil is a uracil analogue that interferes with viral RNA. Synthesis by incorporation might have inhibited virus replication in the present study, resulting in the production of PVY-free plants. Additionally, minimal contamination in potato sprouts was achieved using sodium hypochlorite and ethanol as surface sterilizing agents, which can reduce or eradicate external contaminants. The results showed that ribavirin, as a virucide inducer, reduced the severity of PVY. A virucide may function as an inhibitor of viral replication or create resistance. Physiologically active substances in plant products act as elicitors, creating host plant resistance and slowing disease spread (Daigham *et al*., 2023; Hashem *et al*., 2023b).

The antiviral version of ribavirin triphosphate is thought to work by preventing viral RNA caps from forming. The suppression of PVY is not amenable to this method of action because the protein at the 5'-terminus of the RNA is most likely covalently bound to it (Lerch, 1987).This result was suggested by (Shehata *et al.,* 2023). Contaminants of micro propagated potato shoots were isolated using suitable specific media. The bacterial contaminants were assigned to major genera (*Serratia, Pseudomonas, Bacillus,* and *Xanthomonas*) based on their characteristics. The occurrence rate of bacterial isolates was higher than that of fungal isolates. In the meantime, *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Trichoderma* sp., and *Geotrichum* sp. were the most common fungal pollutants. These findings are consistent with those of (Lata *et al.,* 2006), who revealed a variety of contaminants and microorganisms related to plant tissue cultures. During laboratory modifications of the explant, pollutants could be introduced. 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*. Lactobacillus is an important genus with Generally Regarded As Safe (GRAS) status lactic acid bacteria (Halami *et al.*, 2011; Ołdak and Zielińska, 2017). Certain species within these genera are used as antimicrobial agents to strengthen plant resistance against infections (Abdelaziz *et al.*, 2023c and Hashem *et al.,* 2023a). These include the formation of bacteriocins, hydrogen peroxide, and diacetyl, as well as competitive colonization and the creation of organic acids like lactic and acetic acids (Mishra and Lambert, 1996 and Ouda *et al.*, 2014). It has been observed that lactobacilli create a wide range of bacteriocins, including nisin, lactobrevin, acidophilin, acidolin, lactobacillin, lactocidin, and lactolin (Shahani and Chandan, 1979). The effectiveness of bacteriocins against various food-borne diseases varies based on their specificity (Mishra and Lambert, 1996; Mokoena *et al.,* 2021).

The interaction between bacteriocin and contaminated bacteria and fungi in mixed liquid culture can be bactericidal for those bacteria and fungi. Our study aimed to expand our understanding of the bactericidal impact that mixed Lb. acidophilus produces against contaminated bacteria and fungi during micropropagation of potato plantlet cultures. The screening of microbial contaminants for biotic inducers is based on the reduction of PVY infectivity and potato plantlet growth rates. Most of these, including ribavirin, bacteriocin, and gelrite, have been reported to increase culture mortality. The presence of latent infections can result in variable growth, tissue necrosis, reduced shoot proliferation, and reduced rooting. However, some showed a significant effect on the growth parameters of potato plantlets. Therefore, ribavirin, bacteriocin, and gelrite were chosen to study the effect of their culture filtrate on plantlet growth under tissue culture conditions. In this study, further research was conducted on Lb. acidophilus bacteriocin. The PPE of Lb. acidophilus inhibited contaminated bacteria and fungi during the micropropagation of potato plantlet cultures. This supported previous results on bacteriocin activity against sensitive bacterial species within the same genus (Kang and Lee, 2005). Depending on the strain that produces bacteriocin, the strain that serves as an indicator, and the technique employed to detect bacteriocin, various spectra of inhibitory action may be obtained (Drider *et al.,* 2006). Bacteriocins' recognized method of action on both gram-positive and gram-negative bacteria is their adsorption on cell surfaces, causing pore development. Cell death follows due to electrolyte loss in cells (Alvarez-Cisneros *et al.,* 2011). The data show that Lb. acidophilus produced antibacterial compounds, reducing the number of contaminated bacteria and fungi during micropropagation of potato plantlet cultures from more than 5 log10 CFU to 4 log10 CFU within 24 hours at 37 °C. As described, these experiments demonstrate the bactericidal activity of the Lb. acidophilus mixture against bacteria and fungi in contaminated micropropagated potato plantlet cultures. This phenomenon could have practical applications if it can be performed in vivo.

Meristem tips, 2.0 mm in size, were excised from PVY-infected potato plants cv. sprout under a stereomicroscope. The ratio of persistent diseased plantlets was 85.5%, and PVY elimination was 0.5%. After four subcultures on MS medium supplemented with ribavirin, bacteriocin, and gelrite, the meristems developed into shoots. The survival rates of potato plantlets were 14.3%, 6.7%, 5.0%, and 4.5% for PVY-

infected plantlets, while they were 85.7%, 93.3%, 95.0%, and 95.5% for PVY-free (healthy) plantlets, and 71.4%, 80.0%, 82.8%, and 85.8% for five treatments respectively. These results were confirmed by the DAS-ELISA assay. The present invention relates to an ophthalmic composition comprising at least one active principle and at least one ophthalmically acceptable osmotic agent with antimicrobial activity, typically xylitol, as well as a process for its preparation. The composition may include antiglaucoma drugs, antibiotics, and compounds with antiviral activity. A method of making a surface or substrate antimicrobial involves forming or coating it with compositions that provide antimicrobial, antibacterial, antiviral, and antifungal properties. The Gelrite did not change in viscosity, pH, or temperature.

Regarding the supplemented Ribavirin, bacteriocin, and Gelrite to the MS medium culture, there was an enhancement in all growth parameters (length and number of shoots, roots, and leaves, as well as growth rate) compared to the control (healthy or PVY-infected potato plantlets). It is worth mentioning that Ribavirin, bacteriocin, and Gelrite showed a higher promotion effect on potato plantlets' growth than both healthy and PVY-diseased controls. The results revealed delayed growth in potatoes infected with PVY. The infection caused by PVY resulted in decreased plant height, leaf area, fresh and dried weights, and tuber production. Disruptions in the distribution or supply of hormones that regulate growth may be linked to decreased host plant development (Guo *et al.*, 2000). Potato plants with viral infections exhibit reduced growth in their shoots and roots, leading to lower photosynthetic activity, which directly correlates with yield and product quality. Similar outcomes were observed by (Khalimi and Suprapta, 2011).

The obtained data revealed that bacteriocin and/or gelrite inducers increased the protein content and oxidative enzyme activities of potato plantlets in vitro. They were compared with healthy and PVY-infected plantlets. PVY infection led to a reduction in Chl a, Chl b, and carotenoid contents compared to healthy potato plantlets. Generally, potato plantlets grown on MS medium supplemented with bacteriocin and/or gelrite showed an increase in Chl a, Chl b, and carotenoid contents compared to infected ones. A biotic inducer caused an increase in salicylic acid content in PVY-infected potato plantlets compared to healthy ones. The reduction in carotenoids and chlorophyll in PVY-infected potato plantlets may result from ROS damaging the chlorophyll and thylakoid membrane, preventing the plant from absorbing light and consequently causing photosynthesis to slow down or cease (Abdelaziz *et al.,* 2022). Inhibition of ethylene production, disruption of membrane depolarization, and an increase in photosynthetic rate and chlorophyll content in potato leaves were among the effects of virucides (Ribavirin, bacteriocin, and Gelrite) as a regulatory role in plant physiology. Due to its richness in macro- and microelements, phytohormones, and vitamins, it can be regarded as an effective bio-elicitor containing the necessary elements for cell division, cell elongation, and the generation of photosynthetic pigments. These outcomes align with the research conducted by (Ghobrial *et al.,* 2009).

Expressed qualitative and quantitative protein-related treatments were assayed by SDS-PAGE, where new bands of expressed proteins with molecular weights of 75, 60, 58, 45, and 35 kDa were observed. These are related to ribavirin, bacteriocin, and Gelrite for infected potato plantlets. The production of the protein coat, protein-related virus infection (PR), expressed protein, and other virus-associated non-structural proteins may be the cause of the elevated protein in PVY-infected plantlets.

Furthermore, compared to the untreated plants, virucide treatment significantly increased the amount of soluble protein in both healthy and PVY-infected plants. According to reports, plants undergo various intricate processes involving the synthesis and accumulation of new soluble proteins, which either can directly or indirectly contribute to the development of plant resistance (Abdelaziz *et al.*, 2023b and Abd Alhakim *et al.*, 2022). It has been proposed that the generated proteins could help limit virus propagation or multiplication (Chen *et al.,* 2006). While the presence of a negligible quantity of induced proteins is a prerequisite for the observed systemic infection, the ongoing buildup of newly induced proteins may aid in the localization of viral infection. Drawing from the current understanding of the biochemistry of resistance it may be inferred that SAR is caused by the expression of many factors, such as modifications to the structure of the cell wall and the de novo synthesis of phytoalexins and pathogenesis-related proteins. Furthermore, the induced resistance stage is frequently linked to the de novo synthesis of phytoalexins (Walters *et al.,* 2007). According to reports, one way plants defend themselves against pathogens is by inducing enzymes that activate the phenylpropanoid pathway. This process not only affects the amounts of phenol compounds in the metabolic pool but also creates chemical and/or mechanical barriers in the plant host (Abdelaziz *et al.,* 2023a).

It was shown that PR-1 exhibited enzymatic activity, including glucanase and chitinase, which accumulated proteinase inhibitors and heightened viral resistance. Protein PR-1 biosynthesis was discovered to occur in response to virucide application. SDS-PAGE was used to assess the antiviral proteins' activity, composition, and quantitative aspects, as well as their patterns and content. The outcomes showed that distinct virucide

treatments created different numbers and densities of bands, in addition to a novel pattern of proteins. It has been proven that the induced patterns could prevent the virus from spreading or multiplying (Ahlquist, 2002).

CONCLUSION

Results of the study proved that using high concentrations of ribavirin and bacteriocin during in-vitro regeneration of plantlets from nodal explants could efficiently produce PVY-free plants, although they somewhat affected shoot regeneration.

One of the most significant obstacles that destroy potato tissue culture during the micro-propagated stages is contamination with fungi and bacteria. In this study, the incorporation of ribavirin and/or bacteriocin into the MS medium minimized the number of contaminating bacteria and fungi. This promoted plant development by considerably boosting plant height, leaf size, branch number, and root number. The addition of ribavirin and/or bacteriocin to the MS medium resulted in the growth of PVY virus-free potato seedlings. As a result, the current study suggests integrating ribavirin and/or bacteriocin into an MS medium to increase the quality of potato plants grown in tissue culture. Ribavirin and bacteriocin treatments were found effective. The protocol developed in this study is simple and effective for eradicating PVY from infected potato plants. This technique would be used for the production of PVY-free potato seed stock.

Acknowledgements: The authors want to give thanks to the Botany and Microbiology Department, Faculty of Science, Al-Azhar University, for advancing this study. Also, the authors thank the Virus and Phytoplasma, Dept., Plant Pathology Institute, ARC, Giza, Egypt**.** Also, the authors thank the Botany and Microbiology, Dept., Fac. Sci. Banha Univ., Egypt**.**

REFERENCES

Abd Alhakim, A., Hashem, A., Abdelaziz, A. M., & Attia, M. S. (2022). Impact of plant growth promoting fungi on biochemical defense performance of tomato under fusarial infection. *Egyptian Journal of Chemistry*, *65*(13), 291-301.

Abdelaziz, A. M., Attia, M. S., Salem, M. S., Refaay, D. A., Alhoqail, W. A., & Senousy, H. H. (2022). Cyanobacteria-mediated immune responses in pepper plants against fusarium wilt. *Plants*, *11*(15), 2049.

Abdelaziz, A. M., El-Wakil, D. A., Hashem, A. H., Al-Askar, A. A., AbdElgawad, H., & Attia, M. S. (2023a). Efficient role of endophytic *Aspergillus terreus* in biocontrol of *Rhizoctonia solani* causing damping-off disease of *Phaseolus vulgaris* and *Vicia faba*. *Microorganisms*, *11*(6), 1487.

Abdelaziz, A. M., Hashem, A. H., El-Sayyad, G. S., El-Wakil, D. A., Selim, S., Alkhalifah, D. H., & Attia, M. S. (2023b). Biocontrol of soil borne diseases by plant growth promoting rhizobacteria. *Tropical Plant Pathology*, *48*(2), 105-127.

Abdelaziz, A. M., Sharaf, M. H., Hashem, A. H., Al-Askar, A. A., Marey, S. A., Mohamed, F. A., Abdelstar, M. N., Abdelgawad, H., & Attia, M. S. (2023c). Biocontrol of Fusarium wilt disease in pepper plant by plant growth promoting *Penicillium expansum and Trichoderma harzianum. Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *51*(3), 1-23.

Ahlquist, P. (2002). RNA-dependent RNA polymerases, viruses, and RNA silencing. *Science*, *296*(5571), 1270-1273.

Zhang, A. L., Bettoni, J. C., Shi, X., Liu, Y., Yang, B., & Liu, Z. (2024). In vitro chemotherapy-based methods for virus elimination from *Actinidia macrosperma*. *Scientia Horticulturae*, *337(1)*, 113543.

Alvarez-Cisneros, Y. M., Sáinz Espuñes, T. R., Wacher, C., Fernandez, F. J., & Ponce-Alquicira, E. (2011). Enterocins: Bacteriocins with applications in the food industry. *Science against Microbial Pathogens: Communicating Current Research and Technological Advances. 2nd ed.(Ed: A. Méndez-Vilas), Formatex Research Center, Badajoz*, 1112-1123.

Attia, M. S., Elsayed, S. M., Abdelaziz, A. M., & Ali, M. M. (2023a). Potential impacts of *Ascophyllum nodosum*, *Arthrospira platensis* extracts and calcium phosphite as therapeutic nutrients for enhancing immune response in pepper plant against Fusarium wilt disease. *Biomass Conversion and Biorefinery*, *14*(16), 19613-19622.

Attia, M. S., Salem, M. S., & Abdelaziz, A. M. (2022). Endophytic fungi *Aspergillus* spp. reduce fusarial wilt disease severity, enhance growth, metabolism and stimulate the plant defense system in pepper plants. *Biomass Conversion and Biorefinery,* 1-11.

Attia, M. S., Sharaf, M. H., Hashem, A. H., Mahfouz, A. Y., Daigham, G. E., Al-Askar, A. A., Abdelgawad, H., Thabet, A. E., Abdalmohsen, M. M., & Eladly, Y. R. (2023b). Application of *Rhizopus microsporus* and *Aspergillus oryzae* to enhance the defense capacity of eggplant seedlings against Meloidogyne incognita. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *51*(3), 1-23.

Benčić, P., Keppler, M., Kuge, M., Qiu, D., Schütte, L. M., Häner, M., Strack, K., Jessen, H. J., Andexer, J. N., & Loenarz, C. (2023). Non-canonical nucleosides: Biomimetic triphosphorylation, incorporation into mRNA and effects on translation and structure. *The FEBS Journal*, *290*(20), 4899-4920.

Central Agency for Public Mobilization and Statistics. (2018). Estimates by Governorate 1/1/2018. Available online: www.capmas.gov.eg (accessed on November 2, 2018).

Chen, M., QIU, D. W., LIU, Z., YANG, X. F., & CAO, K. Q. (2006). Inhibition of plant activator protein on RNA replication and coat protein synthesis of Tobacco mosaic virus. *Chinese Journal of Biological Control*, *22*(1), 63.

Chikindas, M. L., Weeks, R., Drider, D., Chistyakov, V. A., & Dicks, L. M. (2018). Functions and emerging applications of bacteriocins. *Current opinion in biotechnology*, *49*, 23-28.

Clark, M. F., & Adams, A. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of general virology*, *34*(3), 475-483.

Coseteng, M. Y., & Lee, C. Y. (1987). Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *Journal of Food Science*, *52*(4), 985-989.

Daigham, G. E., Mahfouz, A. Y., Abdelaziz, A. M., Nofel, M. M., & Attia, M. S. (2024). Protective role of plant growth-promoting fungi *Aspergillus chevalieri* OP593083 and *Aspergillus egyptiacus* OP593080 as biocontrol approach against Alternaria leaf spot disease of *Vicia faba* plant. *Biomass Conversion and Biorefinery*, *14*(18), 23073-23089.

Dawson, W. O., & Lozoya, S. H. (1984). Examination of the mode of action of ribavirin against tobacco mosaic virus. Intervirology, 22, 77-84.

De Man, J. D., Rogosa, D., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Microbiology*, *23*(1), 130-135.

Drider, D., Fimland, G., Héchard, Y., McMullen, L. M., & Prévost, H. (2006). The continuing story of class IIa bacteriocins. *Microbiology and molecular biology reviews*, *70*(2), 564-582.

Ezekiel, R., Singh, N., Sharma, S., & Kaur, A. (2013). Beneficial phytochemicals in potato—a review. *Food Research International*, *50*(2), 487-496.

Ferron, F., Decroly, E., Selisko, B., & Canard, B. (2012). The viral RNA capping machinery as a target for antiviral drugs. *Antiviral research*, *96*(1), 21-31.

Food and Agriculture Organization. (2021). Crop water information; Food and Agriculture Organization of the United Nations. Available online: https://www.fao.org/land-water/databases-and-software/crop-information/en/ (accessed on November 7, 2021).

Fraser, R. S. S., & Gerwitz, A. (1984). Effects of 2-α-hydroxybenzylbenzimidazole on tobacco mosaic virus and host RNA synthesis in tobacco leaf discs and plants. *Plant Science Letters*, *34*(1-2), 111-117.

Ghobrial, W. N., Mehesen, A. A., Abass, J. M., Shalaby, M. E., & Omar, A. F. (2009). Potential impacts of Rhizobium and compost tea enriched with rhizobacteria for enhancing protection of faba bean against broad bean mottle virus (BBMV). *Journal of Agricultural Research, Kafer El-Sheikh University*, *35*, 1-25.

Guo, A., Salih, G., & Klessig, D. F. (2000). Activation of a diverse set of genes during the tobacco resistance response to TMV is independent of salicylic acid; induction of a subset is also ethylene independent. *The Plant Journal*, *21*(5), 409-418.

Halami, P. M., Badarinath, V., Manjulata Devi, S., & Vijayendra, S. V. N. (2011). Partial characterization of heat-stable, antilisterial and cell lytic bacteriocin of *Pediococcus pentosaceus* CFR SIII isolated from a vegetable source. *Annals of Microbiology*, *61*, 323-330.

Hanafy, M. S., Matter, M. A., Asker, M. S., & Rady, M. R. (2016). Production of indole alkaloids in hairy root cultures of *Catharanthus roseus* L. and their antimicrobial activity. *South African Journal of Botany*, *105*, 9-18.

Hashem, A. H., Abdelaziz, A. M., & Attia, M. S. (2023a). Trichoderma a Promising Biofungicide. In *Biofungicides: Eco-Safety and Future Trends* (pp. 166-189). CRC Press.

Hashem, A. H., Attia, M. S., Kandil, E. K., Fawzi, M. M., Abdelrahman, A. S., Khader, M. S., Khodaira, M. A., Emam, A. E., Goma, M. A., & Abdelaziz, A. M. (2023b). Bioactive compounds and biomedical applications of endophytic fungi: a recent review. *Microbial Cell Factories*, *22*(1), 107.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2008). *Modern food microbiology*. Springer Science & Business Media.

Kang, J. H., & Lee, M. S. (2005). Characterization of a bacteriocin produced by Enterococcus faecium GM-1 isolated from an infant. *Journal of Applied Microbiology*, *98*(5), 1169-1176.

Khalimi, K., & Suprapta, D. N. (2011). Induction of plant resistance against Soybean stunt virus using some formulations of Pseudomonas aeruginosa. *Journal of ISSAAS (International Society for Southeast Asian Agricultural Sciences)*, 17(1), 98-105.

Khattab, A. A., Alsherif, W., & El-Masry, S. S. (2021). Improvement of chicken's immunity against velogenic newcastle virus-associated feeding on probiotics. *Egyptian Academic Journal of Biological Sciences, G. Microbiology*, *13*(2), 111-126.

Khattab, A. M., Abo-Taleb, H. A., Abdelaziz, A. M., El-Tabakh, M. A., El-Feky, M. M., & Abu-Elghait, M. (2022). *Daphnia magna* and *Gammarus pulex*, novel promising agents for biomedical and agricultural applications. *Scientific Reports*, *12*(1), 13690.

Kreuze, J. F., Souza-Dias, J. A. C., Jeevalatha, A., Figueira, A. R., Valkonen, J. P. T., & Jones, R. A. C. (2020). Viral diseases in potato. *The potato crop: its agricultural, Nutritional and Social Contribution to Humankind*, 389-430.

Lata, H., Li, X. C., Silva, B., Moraes, R. M., & Halda-Alija, L. (2006). Identification of IAA-producing endophytic bacteria from micropropagated Echinacea plants using 16S rRNA sequencing. *Plant cell, Tissue and Organ Culture*, *85*, 353-359.

Leifert, C., & Cassells, A. C. (2001). Microbial hazards in plant tissue and cell cultures. *In Vitro Cellular & Developmental Biology-Plant*, *37*, 133-138.

Lerch, B. (1987). On the inhibition of plant virus multiplication by ribavirin. *Antiviral research*, *7*(5), 257-270.

Loake, G., & Grant, M. (2007). Salicylic acid in plant defense—the players and protagonists. *Current opinion in plant biology*, *10*(5), 466-472.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*(1), 265-275.

Macaluso, G., Fiorenza, G., Gaglio, R., Mancuso, I., & Scatassa, M. L. (2016). In vitro evaluation of bacteriocin-like inhibitory substances produced by lactic acid bacteria isolated during traditional Sicilian cheese making. *Italian Journal of Food Safety*, *5*(1).

Mahmood, A., & Ali, S. (2017). Microbial and viral contamination of animal and stem cell cultures: common contaminants, detection and elimination. *Journal of Stem Cell Research and Therapy, 2*(5), 1-8.

Malik, C. P., & Singh, M. (1980). Plant enzymology and histo-enzymology. Kalyani Publications.

Mathur, H., Field, D., Rea, M. C., Cotter, P. D., Hill, C., & Ross, R. P. (2017). Bacteriocin-antimicrobial synergy: a medical and food perspective. *Frontiers in Microbiology*, *8*, 1205.

Mishra, C., & Lambert, J. (1996). *Production of antimicrobial substances by probiotics. Asian Pacific Journal of Clinical Nutrition, 5 (1), 20-24*.

Mokoena, M. P., Omatola, C. A., & Olaniran, A. O. (2021). Applications of lactic acid bacteria and their bacteriocins against food spoilage microorganisms and foodborne pathogens. *Molecules*, *26*(22), 7055.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473-497

Ołdak, A., & Zielińska, D. (2017). Bacteriocins from lactic acid bacteria as an alternative to antibiotics. *Advances in Hygiene and Experimental Medicine,* 71, 328-338.

Ouda, S. M., Debevere, J., & Enan, G. (2014). Purification and biochemical characterization of plantaricin UG1: a bacteriocin produced by Lactobacillus plantarum UG1 isolated from dry sausage. *Life Science Journal, 11*(8), 271-279.

Ram, R., Verma, N., Singh, A. K., Singh, L., Hallan, V., & Zaidi, A. A. (2005). Indexing and production of virus-free chrysanthemums. *Biologia Plantarum*, *49*, 149-152.

Shahani, K. M., & Chandan, R. C. (1979). Nutritional and healthful aspects of cultured and culture-containing dairy foods. *Journal of Dairy Science*, *62*(10), 1685-1694.

Shehata, R. S., Moawod, H., & Dawoud, R. A. (2023). Microbial Filtrates Improved Growth Parameters of In vitro PVX Infected Potato Plantlets. *International Journal of Plant and Soil Science*, *35*(20), 711-717.

Simpkins, I., Walkey, D. G. A., & NEELY, H. A. (1981). Chemical suppression of virus in cultured plant tissues. *Annals of Applied Biology*, *99*(2), 161-169.

Studier, F. W. (1973). Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *Journal of molecular biology*, *79*(2), 237-248.

Tamisier, L., Szadkowski, M., Girardot, G., Djian-Caporalino, C., Palloix, A., Hirsch, J., & Moury, B. (2022). Concurrent evolution of resistance and tolerance to potato virus Y in *Capsicum annuum* revealed by genome-wide association. *Molecular Plant Pathology*, *23*(2), 254-264.

Tsai, C. H., Lee, P. Y., Stollar, V., & Li, M. L. (2006). Antiviral therapy targeting viral polymerase. *Current pharmaceutical design*, *12*(11), 1339-1355.

Walters, D. R., Newton, A. C., & Lyon, G. D. Induced Resistance for Plant Defense. Wiley Online Library.

Ziv, M. (1992). The use of growth retardants for the regulation and acclimatization of in vitro plants. In *Progress in Plant Growth Regulation: Proceedings of the 14th International Conference on Plant Growth Substances, Amsterdam, 21–26 July, 1991* (pp. 809-817). Dordrecht: Springer Netherlands.