

Bio-stimulatory and elicitor effects of *Fusarium oxysporum* B17 culture filtrate on In vitro growth, biochemical responses, and genetic stability on Sewi cultivar of date palm (*Phoenix dactylifera* L.) during the germination stage in tissue culture

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Abstract

Date palm is considered one of the most important fruit crops in many regions due to its high nutritional, economic, and ecological value. This study aimed to use cell-free culture filtrate (CFCF) from the fungus *Fusarium oxysporum* B17 isolate (non-fusaric acid producing) as a fungal elicitor to improve date palm in tissue culture. Fumonisin mycotoxins in CFCF were assayed using LC–MS/MS and results confirmed the absence of mycotoxins in CFCF. In addition to that, results for the determination of cytokines in CFCF using GS-MS/MS referred to 6-Benzyl adenine (BA) and Kinetin were present with concentration 0.23%, and 0.64% respectively. Some biochemicals in CFCF were determined, including total indol, amino- acids, phenol, antioxidants, flavonoids, and indol acetic acid (IAA), and gibberellins (GA3), which were 0.13mg/g, 0.217mg/g, 13.54ppm, 87.67%, 1.97mg/g, 0.03mg/g. and 42.04mg/g, respectively. CFCF was applied to the culture medium in two forms: autoclaved and filtered using a syringe filter 0.45µm. Both forms were added to the media with several concentrations (15, 20, 25, and 30%) to plantlets sewi cultivar at germination stage. The results showed the treated plantlets with filtrated with concentration 15% get the significant results in the number of shoots and embryos. Biochemical in treated plantlets was assayed, and the high significant level of each of total phenol, indols, amino-acids, GA3, flavonoids, and IAA was in treated plantlets with 15% of CFCF. Genetic stability of the treated plantlets was tested using ISSR-PCR using 10 primers. The obtained banding patterns were completely monomorphic among all treated and control.

Key message

Cell-free culture filtrate of *F.oxysporum* B17 was used as fungal elicitor to improve date palm plantlets and did not affect on plant's genome.

1. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) considered the most significant fruit crops in semi-arid and arid areas. In the North Africa and Middle East, date palm plays a major role in food security, sustainable agriculture, and rural livelihoods (**Soomro and Shaikh, 2023**). In many nations, date fruits are a staple rich with vital nutrients like fiber, minerals, vitamins, and carbs. For those who reside in areas with few agricultural resources, they are a very vital source of nourishment and energy (Oladzad et al., 2021). Due to its high level of carbs (about 70%), various minerals (such as potassium, iron, and calcium), proteins (2–5%), a complex of vitamins (A, B1, B2, and C), and oil (0.2–0.5%), date fruits are a source of sustenance for people all over the world (Lewaa and Zakaria, 2023)

Tissue culture technique (as a modern technique) in date palm using offshoots and immature female inflorescences is considered the economic propagation protocol. In particularly immature female

inflorescences are successfully used to propagate distinguished and rare cultivars of date palm (Malhat et al., 2019).

In recent years, microbes (in particularly fungi and bacteria) generated secondary metabolites which used as bio-stimulants and elicitors in plant tissue culture and crop enhancement systems. The application of these productions has received more attention (Asim et al., 2022). Cell free culture filtrate (CFCF) from microbes have demonstrated encouraging effects on plant development, morphogenesis, and the accumulation of important biochemical elements, especially those obtained from fungal culture filtrates (Cui et al., 2024). A complex variety of bioactive secondary metabolites, including as phenolic compounds, flavonoids, amino acids, antioxidants, and phytohormones, may be present in these filtrates and have the potential to affect the physiological and developmental responses of plants (Zhao et al., 2019).

Fusarium genera consider one of important fungi that have ability to synthesize biologically active metabolites, including indole-3-acetic acid (IAA), gibberellins, and other hormone-like compounds that may function as plant growth regulators. Some isolates of *Fusarium oxysporum* have been reported to produce phytohormone-related metabolites and to act as plant growth-promoting fungi under specific conditions. This highlights the possibility of utilizing selected non-harmful fungal metabolites as elicitor-like agents to improve plant growth and biochemical performance *In vitro* (Maan and Abdelhamid, 2026).

Therefore, the present study was designed to biochemically and hormonally characterizes the crude culture filtrate of *Fusarium oxysporum* and to evaluate its role as a fungal elicitor in date palm tissue culture. In addition, the effects of both filtered and autoclaved filtrate fractions at different concentrations on the biochemical responses of treated plantlets were investigated. The study also aimed to verify the genetic stability of treated and untreated regenerants using ISSR markers, in order to determine whether fungal elicitation could improve physiological performance without inducing detectable genetic variation.

2. MATERIALS AND METHODS

This study's experiments were conducted at the Central Laboratory for Date Palm Research and Development (CLDPRD) and the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt.

2.1. Plant materials

The explants in the emryo germination stage, which were obtained from immature female Sewi cultivar inflorescences (5–7 cm) that were collected from a farm in Abu-Suwir, Ismailia Governorate, Egypt, located at 32°11'20.6"E 30°29'37.3"N were cultivated on MS medium according to Murashige and Skoog (1962). The explants (indirect somatic cluster 2–3 embryos) were cultured after that according to.

Malhat et al. (2019) protocol as follows: state that 100 mg/l glutamine, 100 mg/l myoinositol, 1 mg/l biotin, 5 mg/l thiamin HCl, 40 g/l sucrose, and 6 g/l agar were added to MS media. After adjusting the pH to 5.7–5.8, it was sterilized for 20 minutes at 121°C and 15 lbs/ins. Every cultured explant was kept at $27 \pm 2^\circ\text{C}$ in a controlled growing chamber. Explants were grown from stages of initiation, maturation, and germination to be ready to use.

2.2. Source of fungus

Fusarium oxysporum B17 isolate (non-fusaric acid producing) was graciously provided by Dr. Eman A. Elkamshoush of the Department of Medicinal and Aromatic Plant Research at the Horticulture Research Institute, ARC.

2.3. Preparation of Cell-Free Culture Filtrate (CFCF)

The isolate of *Fusarium oxysporum* B17 was maintained on Potato Dextrose Agar (PDA) at 4°C and subcultured prior to experimentation. For culture filtrate preparation, mycelial discs (about 8 mm in diameter) were transferred under aseptic condition into 500 mL Erlenmeyer flasks that containing 100 mL of sterile Potato Dextrose Broth (PDB). The flasks then were incubated at $28 \pm 2^\circ\text{C}$ on a rotary shaker at 160 rpm for 10 days to allow sufficient fungal growth and extracellular secretion of metabolite. Following the incubation, the biomass of fungi was excluded from the liquid medium by filtration using sterile Whatman™ No. 1 filter paper. Subsequently, the filtrate was centrifuged at 8,000 rpm for 15 min at 4°C to remove any residual of fungal hyphae fragments and fine particulate matter. The supernatant was collected carefully under aseptic conditions and considered as the crude cell-free culture filtrate. This method was carried out according to Bhatt and Jampala (2020).

2.4. Sterilization Treatments and Media Preparation

To evaluate the effect of heat exposure on the biological activity of the fungal metabolites, the CFCF was divided into two portions: Membrane-filtered treatment: One portion was sterilized by passage through a $0.45 \mu\text{m}$ sterile syringe filter and subsequently added aseptically to previously autoclaved and cooled culture media. Heat-treated treatment: The second portion was directly incorporated into the culture medium prior to sterilization. The medium supplemented with the filtrate was then autoclaved at 121°C for 20 min. A control treatment consisting of medium without culture filtrate was prepared under identical conditions.

2.5. Determination of fumonisins in CFCF by LC-MS/MS

According to Sulyok et al., (2020) method, The CFCF obtained from *Fusarium oxysporum* B17 was subjected to quantitative chemical analysis to determine the presence and concentration total fumonisins (FB_1 and FB_2) as the myco-toxin which excreted from *Fusarium* spp.

Quantitative determination of total fumonisins (sum of FB_1 and FB_2) was carried out at the Central Laboratory for Residue Analysis of Pesticides and Heavy Metals in Food, Agricultural Research Center, Ministry of Agriculture, Egypt (Giza, Egypt).

2.5.1. Sample preparation

Aliquots of the CFCF were filtered through a 0.22 µm membrane filter prior to analysis. Where necessary, samples were diluted with LC–MS grade methanol–water solution (typically 50:50, v/v). For improved analytical accuracy, matrix-matched calibration curves were prepared using certified reference standards of fumonisins

2.5.2. Fumonisins determination

Analysis was performed using a triple quadrupole liquid chromatography–tandem mass spectrometry (LC–MS/MS) system equipped with an electrospray ionization (ESI) source operating under multiple reactions monitoring (MRM) mode for targeted quantification. Chromatographic separation was achieved using a reversed-phase analytical column under gradient elution conditions optimized for fumonisin detection.

Quantification was conducted following a validated LC–MS/MS protocol for total fumonisins. Calibration curves were prepared using certified analytical standards, and analyte concentrations were calculated based on external standard calibration.

The limit of quantification (LOQ) for fumonisins B₁ and B₂ was 40 µg kg⁻¹. Measurement uncertainty, expressed as expanded uncertainty at a 95% confidence level, was within ± 30%, as reported by the accredited laboratory. The analysis was conducted by an ISO-accredited laboratory following validated international protocols and the results were issued under test certificate No. 1707051

2.6. Biochemical production assessment

Some of biochemical production from *F.oxysporum* B17 in CFCF was assayed. Total phenol, Total indol, Total amino acids, Gibberellins (GA3), Antioxidant activity, Total flavonoids, and Indol acetic acid (IAA) were assayed spectroscopy using spectrophotometer as mentioned later while cytokinesis were assayed using GC-MS/MS

2.7. Screening for cytokinesis

Cytokinesis was assayed in CFCF to investigate the presence of putative plant growth regulators (PGRs). The protocol described by Santana et al., (2013) using GC-MS/MS as follows:

2.7.1. Sample Preparation

For GC–MS analysis, aliquots of the filtrate were concentrated under reduced pressure or a gentle nitrogen stream at controlled temperature. Due to the polar and semi-volatile nature of many phytohormone-like compounds, samples were subjected to derivatization when required to enhance volatility and chromatographic performance.

2.7.2. GC–MS/MS analysis

Chemical profiling was carried out using a Gas Chromatography system (Agilent Technologies 7890A) coupled to a Mass Selective Detector. Separation was achieved using an HP-5ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) (Carrier gas: Helium (constant flow ~ 1.0 mL min⁻¹), Injection mode: Split, depending on concentration Oven temperature program: Gradient heating from low initial temperature to ~ 280°C, Ionization mode: Electron ionization (EI) at 70 eV.

Mass spectra were recorded in full scan mode (m/z 50–600) for compound screening. Putative identification of hormone-like compounds was performed by comparison of retention times and spectral fragmentation patterns with authentic standards and NIST mass spectral libraries. This qualitative screening allowed detection of potential growth-promoting compounds, including cytokinin-like, auxin-like, or other bioactive metabolites, without restricting the analysis to predefined targets.

Determination of growth regulators in CFCF was conducted at the Regional Center for Food and Feed (RCFF), Agricultural Research Center, Giza, Egypt, an accredited governmental laboratory, and the results were officially documented under Certificate No. 665.

2.8. Effect of CFCF on the growth of somatic embryos

Generally, clusters of somatic embryos (three to four embryos) were employed as explant materials during the germination stage. According to Bhatt and Jampala (2020), the explants were cultured in jars that included 20ml of MS media that had different concentrations of CFCF (15, 20, 25, and 30%) and the control to evaluate its biological effect in a dose-dependent manner. The explants were cultivated. A controlled growth chamber was used to incubate the treated jars at 27 ± 2. The °C

The CFCF was split into two parts in order to evaluate how sterilization affected the stability of the metabolites.

2.8.1. Autoclaved CFCF treatment

Prior to sterilization, a measured volume of crude CFCF was added to the culture medium to achieve final concentrations of 15%, 20%, 25%, and 30% (v/v). After that, the extract-containing medium was heat-sterilized for 20 minutes at 121°C and 15 pressure. The purpose of this treatment was to assess how the CFCF components affected plant growth.

2.8.2. Filter-sterilized extract treatment

The second portion of the extract was sterilized by membrane filtration using a 0.45 µm syringe filter. To obtain the same final concentrations (15%, 20%, 25%, and 30% v/v), the sterile filtrate was aseptically added to culture medium that had already been autoclaved and semi-cooled. Under order to preserve sterility, the addition was carried out under a laminar airflow. By using this method, putative heat-labile plant growth regulators that might break down during autoclaving were conserved.

Three subcultures of clusters were cultivated at one-month intervals. Lastly, somatic embryos' growth and development during the germination and multiplication stages were observed and documented.

2.9. Biochemical changes

The biochemical change was evaluated using three duplicates of each treatment. Ten milliliters of 80% ethanol were added to one gram of each treatment sample. Each sample was homogenized with a pestle and mortar before being kept for a full day at 4°C in the dark. After centrifuging the sample at 13000xg/5min, the supernatant was transferred to the test for antioxidants, flavonoids, total amino acids, total phenol, total indols, indol acetic acid (IAA), and gibberellins (GA3).

2.9.1. Total phenol

According to Ainsworth and Gillespie (2007) modified Patel et al. (2010), the estimated total phenol contents were as follows: A tube was filled with one milliliter of the previously extracted material, five milliliters of dH₂O, and roughly five hundred microliters of Folin-Ciocalteu reagent (F-C). The mixture was vortexed for five minutes. After that, 1.5 ml of 20% sodium carbonate was added, bringing the total amount to 10 ml. The tubes were incubated for two hours at room temperature in the dark room. Lastly, a spectrophotometer (Orion AquaMate 8000) with a wavelength of 765 nm was used to record the data.

2.9.2. Total indol

According to Selim et al. (1978), the following technique was employed to estimate the total indol contents: Four milliliters of para di-amino benzoate (PDAB) were added to a tube containing one milliliter of the aliquot sample. After that, the tubes were incubated for an hour at 37°C. The Orion AquaMate 8000 spectrophotometer was used to measure the samples at 530 nm.

2.9.3. Total amino acids

As method of **Mc. Grath (1972)**, one milliliter of ninhydrin was added as a reagent following the addition of one milliliter of the extracted sample and four milliliters of deionized water to fill the empty space. The tubes were covered and submerged in water at 100°C for fifteen minutes. One milliliter of 50% ethanol was added after the tubes had been submerged in cooled water. At wavelength 570 nm, the Orion AquaMate 8000 spectrophotometer finally showed red samples.

2.9.4. Gibberellins (GA3)

Gibberellins (GA3) were assayed using Sayed et al. (2019) as follows:

2.9.4.1. Gibberellins extraction

Gibberellins were extracted in the manner described below: After adjusting the filtrate's pH to 2.8 with HCl (1N), it was extracted using ethyl acetate three times, discarding the aqueous phase each time. After gathering the ethyl acetate phase, GA3 was prepared for testing.

2.9.4.2. Gibberellins assaying

A test tube was filled with about 300 microliters of collected ethyl acetate phase, 1 milliliter of HCl (10N), 1 milliliter of Folin-Ciocalteu reagent, 3 milliliters of water, and a thorough vortex. The test tubes were left to cool to room temperature after being immersed in a bath of boiling water for five minutes. Lastly, the spectrophotometer (Orion AquaMate 8000) showed that the samples were red at 750 nm.

2.9.5. Antioxidant activity

Using 2, 2'-diphenylpicrylhydrazyl (DPPH), antioxidant activity in extracted materials was assessed. According to Burits and Bucar (2000), antioxidant activity was calculated as follows:

One milliliter of the extracted sample and one milliliter of 0.004% (w/v) DPPH (dissolved in 95% methanol) were added to the test tube, and the mixture was allowed to sit at room temperature for thirty minutes. Next, using quercetin as a positive control, samples were examined on the Orion AquaMate 8000 spectrophotometer at 517 nm and compared to DPPH (as a blank). The percentage activity of antioxidants (%) was determined using the following formula,:

$$I\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

I%: The percentage of antioxidant activity

A_{blank} : the absorbance of the positive control

A_{sample} , the absorbance of the tested sample

2.9.6. Total flavonoids

According to Quettier-Deleu et al. (2000), total flavonoids were calculated as follows: One milliliter of the extracted material, one milliliter of 2% aluminum chloride dissolved in methanol, and 0.2 milliliters of 1M potassium acetate were added to the test tube. The mixture was red on the Orion AquaMate 8000 spectrophotometer at 430 nm after 30 minutes of room temperature incubation.

2.9.7. Indol acetic acid (IAA)

Indol acetic acid (IAA) was estimated using the method outlined by Glickmann and Dessaux (1995): In a test tube, one milliliter of the extracted sample and one milliliter of the Salkowski reagent (12 g/l of FeCl₃, 7.9M H₂SO₄) were combined, and the combination was left in the dark for thirty minutes. The mixture was used to estimate IAA at a wavelength of 530 nm on the Orion AquaMate 8000 spectrophotometer.

2.10. Genetic stability

Using inter simple sequence repeat (ISSR)-PCR fingerprinting, the stability of the treated explants in the multiplication stage with AgNPs and silver nitrate was assessed at the genetic level.

2.10.1. DNA extraction

According to Arif et al. (2010), the DNA from plant tissue was extracted as follows: 500µl of lysis buffer (100ml/pH 8.0 with 0.4g Na2EDTA, 1.21g tris-base, 8.12g NaCl, 2.0g CTAB, and 2.0g PVP) was used to grind 100 mg of plant tissue in mortar. The pulverized tissue was placed into a 1.5 ml Eppendorf tube and maintained in a water bath at 60°C for 30 minutes. The samples were then centrifuged at 9500xg for five minutes, and the supernatant was moved to a fresh Eppendorf tube. The transferred supernatant was mixed with an equal volume of chloroform:isoamyl-alcohol (24:1), thoroughly vortexed, and then centrifuged at 9500xg/5min. After transferring the aqueous phase to a fresh Eppendorf, 500µl of cool isopropanol and 10% of the transferred volume from 3M potassium acetate were added, gently mixed, and the samples were centrifuged at 11500xg/10min. After discarding the supernatant, samples were centrifuged at 7000xg/5min after 500µl of cold ethanol was added. Before being used, the pellet was stored at 4°C after being resuspended in 100µl of sterile dH2O.

2.10.2. Fingerprinting assays

ISSR primers (10 primers) were used to fingerprint the DNA of the treated plant, as indicated in table (1). The PCR reaction (25µl) comprises 12.5µl of TaqMan (OmniPCR, BIO-HELIX), 6.5µl of dH2O, 2 µl of DNA (~ 250–400ng), and 2 µl of each primer (10 p.mol). The MJ-Research thermo-cycler (PTC-200) was used to perform the PCR amplification protocol, which was 94°C/5min, 35 cycles of 94°C/45sec, 50.5°C/45sec, 72°C/90s, and 72°C/7min. The samples were electrophoresed at 120V for 30 minutes using agarose (1.2%), and the gel was stained with ethidium bromide dye (1 mg/ml).

Table (1):ISSR-primers sequence used in PCR

No.	Primer	Sequence 5'-3'	
1	ISSR-1	AGAGAGAGAGAGAGAGGYC	Lewaa et al., (2024)
2	ISSR-2	AGAGAGAGAGAGAGAGYG	
3	ISSR-3	ACACACACACACACACYT	
4	ISSR-4	ACACACACACACACACYG	
5	ISSR-5	GTGTGTGTGTGTGTGTYG	
6	ISSR-6	CGCGATAGATAGATAGATA	
7	ISSR-7	GACGATAGATAGATAGATA	
8	ISSR-8	AGACAGACAGACAGACGC	
9	ISSR-9	GATAGATAGATAGATAGC	
10	ISSR-10	GACAGACAGACAGACAAT	

2.10.3. Analysis of data

ISSR-PCR amplified fragments were assessed as either present (1) or absent (0), with only distinct fragments receiving a score. The obtained data was analyzed using a matrix of similarity utilizing Jaccard's coefficient using the Past program (version 4.03).

2.11. Statistical analysis of data

In accordance with Hoshmand (2017), all gathered data were statistically analyzed using SPSS version 23 using analysis of variance (ANOVA).

3. RESULTS

3.1. Total fumonisins determination by LC-MS/MS

Quantitative LC–MS/MS analysis revealed that total fumonisins (sum of FB1 and FB2) were not detected in the analyzed samples. The measured concentrations were below the established limit of quantification (LOQ) of 40 µg kg⁻¹, as defined by the accredited analytical method and no characteristic multiple reaction monitoring (MRM) transitions corresponding to fumonisin B1 or B2 were observed above the signal-to-noise threshold required for reliable quantification. Therefore, the samples were considered compliant with the applied analytical criteria and free from detectable levels of fumonisins under the experimental conditions employed.

3.2. Biochemical production assessment

The biochemical production from CFCF revealed the presence of several biologically active compounds. As mentioned in Table (2), the assayed biochemical compounds was as follows: total indol, total amino acid, total phenol, antioxidant, flavonoids, indol acetic acid, and GA3 were (0.13 mg/g, 0.217 mg/g, 13.54 ppm, 87.67%, 1.97 mg/g, 0.03 mg/g, and 42.04 mg/g) respectively.

Table (2): Biochemical and phytohormones composition of CFCF.

Treatment	Estimation
Total indol	0. 13 mg/g
Total amino acid	0.217 mg/g
Total phenol	13.54 ppm
Antioxidant	87.67%
Flavonoids	1.97 mg/g
Indol acetic acid	0.03 mg/g
GA3	42.04 mg/g

3.3. Screening for Cytokinesis

CFCF was analyzed using GS-MS/MS to determine the cytokinesis. From the obtained data, 6-Benzyl adenine (BA) was determined with concentration 0.23%, and Kinetin was determined with concentration 0.64%.

3.4. Effect of CFCF on the germination stage

Growth of embryos and shoots (after 3 subcultures) from the germination stage, which was treated with different concentrations of CFCF (15, 20, 25, and 30%), was divided into two categories (autoclaved CFCF treatment and filter-sterilized extract treatment) were noticed and illustrated in Fig. (1). Results as shown in table (3) and in Fig. (2) referred to the treatments with different concentrations of filter-sterilized extract treatment were better than the treatments with different concentrations of autoclaved CFCF treatment. In addition to that, 15% of filter-sterilized extract treatment was more significant on the number and growth of embryos and shoot formation compared with all treatments and control.

Figure (1): Effects of different concentrations of CFCF on germination Stage A) Control, B) Filtrate (15%), C) Filtrate (20%), D) Filtrate (25%), E) Filtrate (30%), F) Autoclaved (15%), G) Autoclaved (20%), H) Autoclaved (25%), and I) Autoclaved (30%).

Table (3): Effect of CFCF concentrations on number of embryos and shoots in germination stage

Treatment	No. of embryos	No. of shoots
Control	6.0 ^{cd}	2.75 ^{de}
Filtrate 15%	17.5 ^a	13.25 ^a
Filtrate 20%	8.0 ^c	10.25 ^b
Filtrate 25%	4.25 ^d	4.25 ^d
Filtrate 30%	3.75 ^d	4 ^d
Autoclaved 15%	12.25 ^b	8.75 ^b
Autoclaved 20%	7 ^c	6.75 ^c
Autoclaved 25%	3.5 ^d	4 ^d
Autoclaved 30%	4.25 ^d	2.25 ^e
LSD	2.57	1.7

Figure (2): Effect of different concentrations of CFCF of *F.oxysporum* B17 A) on embryo number B) on shoot number

3.5. Bio-chemical assaying

The biochemical (total phenol, total indol, total amino acid, gibberellins, anti-oxidants, total flavonoids, and indol acetic acid) in the explants treated with varying concentrations of CFCF were estimated. As reported in Table (4), and shown in Fig. (3). The culture that treated with concentration 15% of filtrate of CFCF recorded the highest biochemical values for parameters including total phenol (13.01 ppm), total indol (0.1 mg/g), total amino acid (0.551 mg/g), gibberellins (23.19 mg/g), total flavonoids (2.28 mg/g), and indol acetic acid (0.06 mg/g). The results of this treatment were significant with all other treatments except with the culture threated with autoclaved CFCF with concentration 15% in total phenol (12.85 ppm), total amino acid (0.536 mg/g), and indol acetic acid (0.06 mg/g). In case of antioxidant parameter, the highest value was recorded with concentration 30% of filtrate of CFCF (88.92%). On the other hand, the control sample recorded the lowest values in most parameters, in total phenol (3.71 ppm), total indol (0.01 mg/g), gibberellins (7.54 mg/g), antioxidants (81.25%), and indol acetic acid (0.003 mg/g). The results of control sample was significant with other treatments except in total phenol, it was non-significant with autoclaved CFCF with concentration 30% (3.93 mg/g), and in indol acetic acid with filtrated CFCF 25% (0.13 mg/g), and 30% (0.13 mg/g), and autoclaved CFCF with concentration 30% (0.13 mg/g). Finally, the lowest values in total amino acid was noted in both autoclaved CFCF with concentration 25% and 30% (0.22 and 0.197 mg/g) respectively and there was non-significant between them, and in total flavonoids, the lowest value was in the filtrated CFCF 30% (1.04 mg/g) and it was significant with other treatments.

Table (4): Biochemical assaying in treated plants with several concentrations of CFCF

Treatments	Total phenol ppm	Total indol mg/g	Total amino acid mg/g	gibberellins mg/g	Anti-oxidants %	Total flavonoids mg/g	Indol acetic acid mg/g
Control	3.71 ^e	0.01 ^f	0.346 ^d	7.54 ⁱ	81.25 ^h	1.30 ^d	0.003 ^e
Filter 15%	13.01 ^a	0.10 ^a	0.551 ^a	23.19 ^a	83.17 ^g	2.28 ^a	0.060 ^a
Filter 20%	10.85 ^b	0.07 ^b	0.463 ^b	19.72 ^c	86.25 ^d	1.97 ^b	0.030 ^b
Filter 25%	3.85 ^e	0.03 ^e	0.359 ^d	18.59 ^d	86.42 ^c	1.51 ^c	0.013 ^{cde}
Filter 30%	3.66 ^e	0.09 ^b	0.231 ^e	15.22 ^f	88.92 ^a	1.04 ^f	0.013 ^{de}
Autoclaved 15%	12.85 ^a	0.06 ^c	0.536 ^a	20.84 ^b	85.83 ^f	1.59 ^c	0.060 ^a
Autoclaved 20%	9.20 ^c	0.08 ^b	0.427 ^c	17.72 ^e	86.00 ^e	1.19 ^e	0.023 ^{bc}
Autoclaved 25%	4.48 ^d	0.07 ^b	0.220 ^{ef}	14.59 ^g	86.75 ^b	1.23 ^{de}	0.023 ^{bcd}
Autoclaved 30%	3.93 ^e	0.05 ^d	0.197 ^f	10.72 ^h	87.67 ^b	0.92 ^g	0.013 ^{de}
LSD	0.296	0.01	0.0327	0.082	0.1217	0.084	0.012

Figure (3): Effect of different concentrations of CFCF of *F.oxysporum* B17 (filtered and autoclaved) on biochemical constituents including total phenols (A), Total indol compounds (B), Total amino acids (C), Gibberellins (D), Antioxidant activity (E), Total flavonoids (F), Indol acetic acid (G).

3.6. Genetic stability

The genetic stability of explants that treated with CFCF (filtrated and autoclaved) and control were assessed by PCR test. Whole genomic DNA of samples was amplified using 10 ISSR primers. As shown in Fig. (4), all tested samples had the same fragments patterns in the amplification profiles. There were no discernible differences or polymorphic fragments between samples. Since every amplified fragment was monomorphic, the DNA profiles were entirely comparable. From this obtained results, it considered the treatment with CFCF (filtrated and autoclaved) did not affect the genetic stability and did not induce any detectable genetic variation or mutation in the plants

Figure (4): Gel profile for genetic stability for treated samples with several concentrations of CFCF using ISSR-PCR technique A) ISSR-1 and ISSR-2, and B) ISSR-9 and ISSR-10 M: marker (1kb), 1: Control, 2: Filtrate (15%), 3: Filtrate (20%), 4: Filtrate (25%), 5: Filtrate (30%), 6: Autoclaved (15%), 7: Autoclaved (20%), 8: Autoclaved (25%), and 9: Autoclaved (30%).

4. DISCUSSION

Fumonisin myco-toxin is closely related with genus *Fusarium*. It is considered the main reason of isolate virulence during plant infection. It inhibits synthesis of ceramide layer in plant cell wall and causing necrotic lesion, reducing of root elongation, and reducing plant growth. Fusaric acid myco-toxin too which exuded from genus *Fusarium* play an important role in plant infection. It works by affecting the water balance in plant tissues and preventing the formation of roots and shoots.

The absence of fumonisins (FB₁ and FB₂) and fusaric acid, too, in *F.oxysporum* B17, is considered an important and encouraging factor for using this isolate as a natural source of phytohormone and studying the effect of this isolate on date palm explant in tissue culture. Several studies reported that some *Fusarium* isolates have been shown not to produce myco-toxins as mentioned by Desjardins and Plattner (2000) who reported they found *F.verticilloids* non-producing of fumonisins. Bennett et al., (2023) used *F.verticilloids* that non-producing of fumonisins as bio-control agent for other isolates of *F.verticilloids* that producing of fumonisins as cross protection in maize field. Irzykowska et al., (2012) reported that some isolates of *F.oxysporum* did not genetically produce fumonisins myco-toxin.

Some of biochemical (as secondary metabolite) was estimated in cell free culture filtrate (CFCF), we found that total indol was 0.13 mg/g. and indol acetic acid (IAA) was 0.03 mg/g. Total indol compounds are important for plant growth regulation and microbes produced it to make connect with plants to make synergism or infection. Mehmood et al., (2018) referred that IAA in CFCF from *F.oxysporum* was assayed and it was 0.031 mg/g. Also Cheng et al., (2022) reported that *F.oxysporum* KB-3 was used as mycorrhizal fungus because of the IAA ratio which produced and estimated and it was 0.076 mg/g. Similar with Maan and Abdelhamid (2026) mentioned that IAA was 0.024 mg/g in isolate of *F.oxysporum*. Total phenol was 13.54 ppm, antioxidants were 87.67%, flavonoids were 1.97 mg/g, and

The genus *Fusarium* is among the microbes that produce gibberellins. GA3 was estimated in CFCF, and it was 42.04 mg/g. This assessment agreed with Mahmoud et al., (2025) who assayed the GA3 that produced from cocktail of species belong to genus *Fusarium* (*F.solani*, *F.oxysporum*, *F.incarnatum*, and *F.camptoceras*) the ratio was 36.33mg/g. Total phenol, flavonoids, and anti-oxidants were estimated and there were 13.54 ppm, 1.97 mg/g, and 87.67% in our isolate. Similar results were agreed with Ebadi et al., (2024) while they estimated the biochemical in isolated fungi from *Gundelia tourneforti*. The high phenolic compound, total flavonoids and antioxidants were estimated in *Alternaria alternata* (8.2 mg/g, 0.3 mg/g, and 47.1% respectively).

Cytokines play an important role as both compounds are well-documented regulators of cell division, shoot proliferation, and morphogenic responses in plant tissues. CFCF was analyzed using GS-MS/MS to determine the cytokinesis. From the obtained data, 6-Benzyl adenine (BA) was determined with concentration 0.23%, and Kinetin was determined with concentration 0.64%. Sørensen et al., (2018) referred to *Fusarium pseudograminearum* produces 4 types of cytokinesis (fusatin, 8-oxo-fusatin, fusatinic acid, and 8-oxo-isopentenyladenine)

To evaluate the effect of heat exposure on the biological activity of the fungal metabolites, the CFCF was divided into two portions: Membrane-filtered treatment: One portion was sterilized by passage through a 0.45 µm sterile syringe filter and subsequently added aseptically to previously autoclaved and cooled culture media. Heat-treated treatment: The second portion was directly incorporated into the culture medium prior to sterilization. The medium supplemented with the filtrate was then autoclaved at 121°C for 20 min. A control treatment consisting of medium without culture filtrate was prepared under identical conditions. Results showed the CFCF generally enhanced and improved the plantlets comparing with control and the concentration 15% by filtration particularly was the best treatment between all treatments. These results agreed with (Baroja-Fernández et al., 2021) when they used CFCF of *Piriformospora indica* with pepper plants. they reported that the pepper plants were enhanced and improved (root growth and fruit yield) in addition to the nutritional characteristics of the fruits. This is due to fungal CFCF release of diffusible materials that encourage development, such as volatile organic chemicals.

Using the ISSR-PCR technique and 10 ISSR primers, the genetic stability of treated explants with various concentrations of both filtrated and autoclaved CFCF (15, 20, 25, and 30%) was examined. According to the results, there was no genetic difference between the treated and control explants. Several studies have indicated that plants treated with fungi had high genetic stability and were not affected. Oliya et al., (2021) mentioned that the CFCF of *F.oxysporum* which was isolated from the shoot stem of *Vanda cristata* (CSV4), and *Alternaria* sp., which was isolated from the leaf of *Vanda cristata* (PVL5) were used as an elicitor for *Rhynchosyilis retusa* (L.), and the genetic stability was tested using 22 RAPD markers. From their obtained results, the CFCF of fungi did not affected on genetics of treated plants.

5. CONCLUSION

In the conclusion, CFCF of *F.oxysporum* was extracted from the mycelium of fungi and proved this CFCF was free from fumonisins toxins. Cytokinesis in CFCF was estimated using LC-MS/MS and 6-Benzyl adenine (BA) and Kinetin were determined. CFCF was divided into 2 groups (filtrated with syringe filter and autoclaved) and was added to date palm tissue culture with concentrations (15, 20, 25, and 30%) of media. The filtrate CFCF with syringe filter with concentration 15% gave the high significant number of shoots and embryos and gave high values of total phenols, total indols, total amino acids, GA3, total flavonoids, and IAA. Genetic stability of treated plants was confirmed using molecular markers (RAPD/ISSR), which showed monomorphic banding patterns compared with the control plants.

Declarations

Ethics

Not applicable

Competing interests: Authors have declared that no competing interests exist

Consent to participate declaration: Informed consent was obtained from all individual participants included in the study

Consent to publish declaration: All authors approve the manuscript and give their consent for submission and publication

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Author Contribution

L. M. and M.H. wrote the main manuscript text and carried out the practical work of the research. A. M. suggest the main idea of this study

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Figures

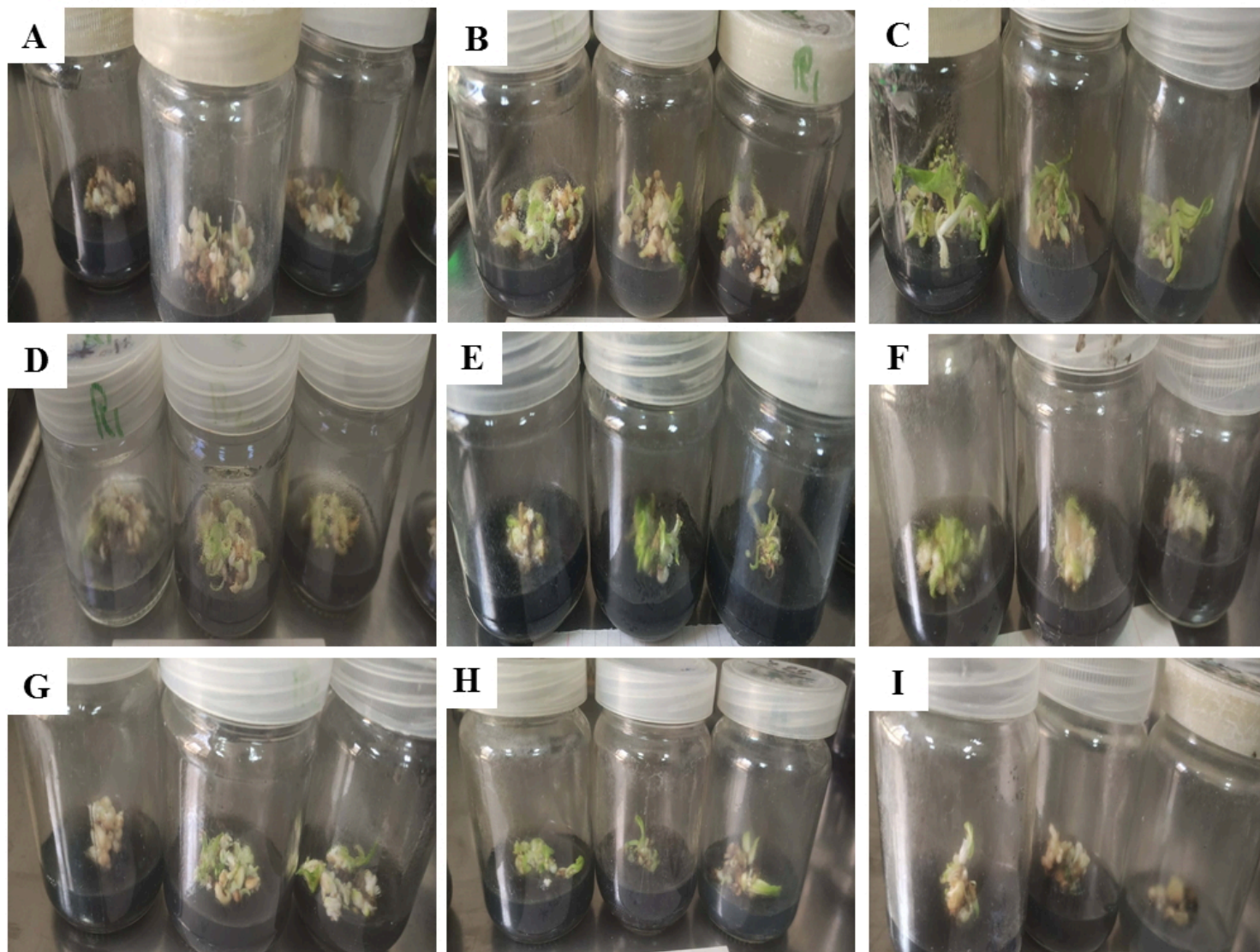


Figure 1

Effects of different concentrations of CFCF on germination Stage A) Control, B) Filtrate (15%), C) Filtrate (20%), D) Filtrate (25%), E) Filtrate (30%), F) Autoclaved (15%), G) Autoclaved (20%), H) Autoclaved (25%), and I) Autoclaved (30%).

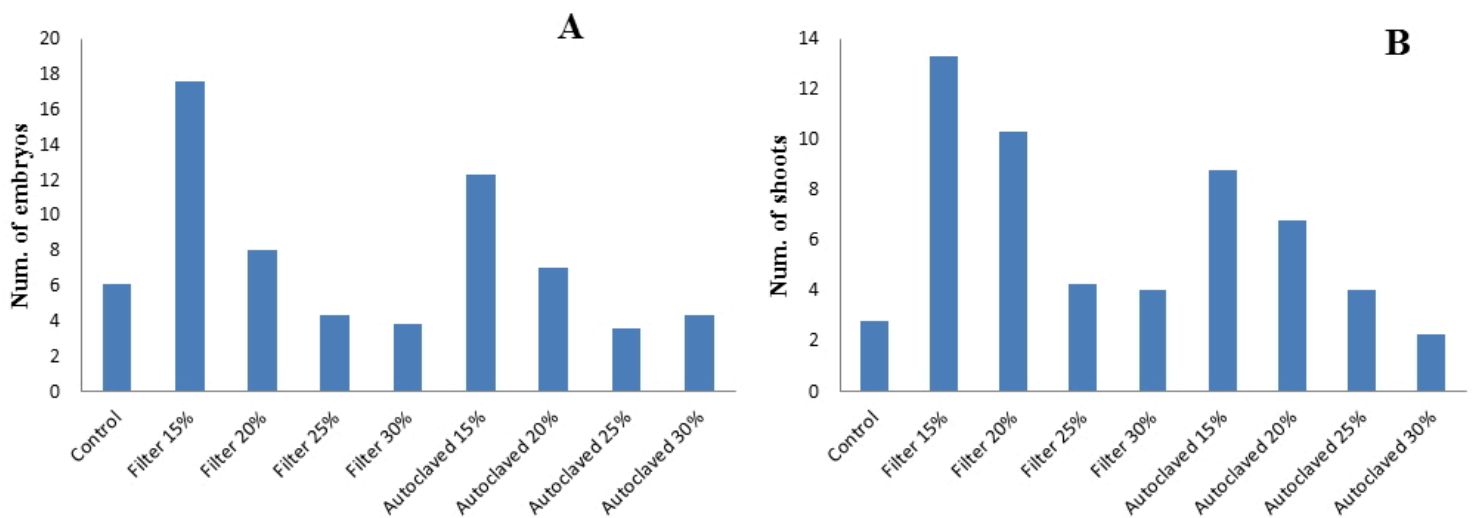


Figure 2

Effect of different concentrations of CFCF of *F.oxysporum* B17 A) on embryo number B) on shoot number

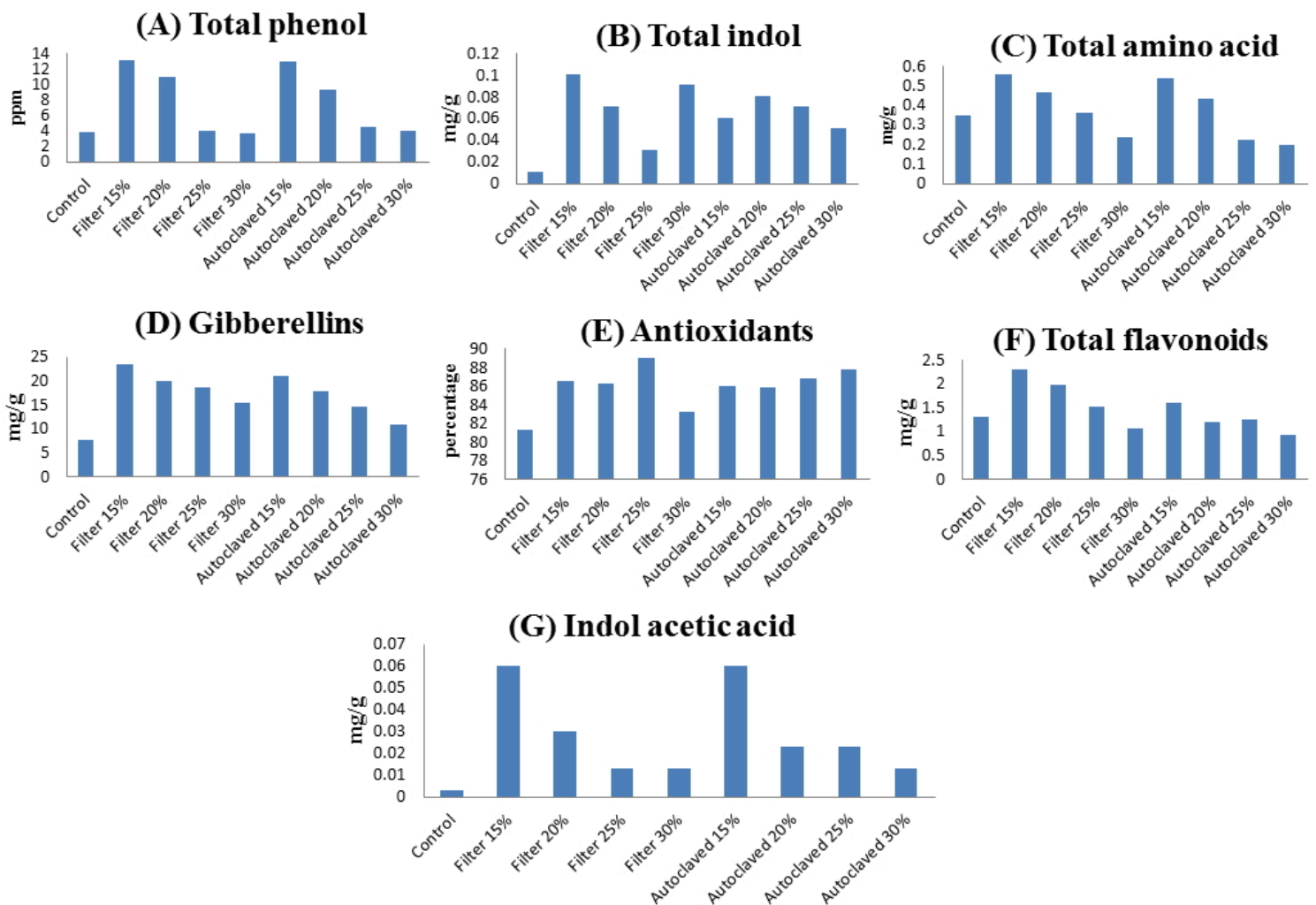


Figure 3

Effect of different concentrations of CFCF of *F.oxysporum* B17 (filtered and autoclaved) on biochemical constituents including total phenols (A), Total indol compounds (B),Total amino acids (C), Gibberellins (D), Antioxidant activity (E), Total flavonoids (F), Indol acetic acid (G).

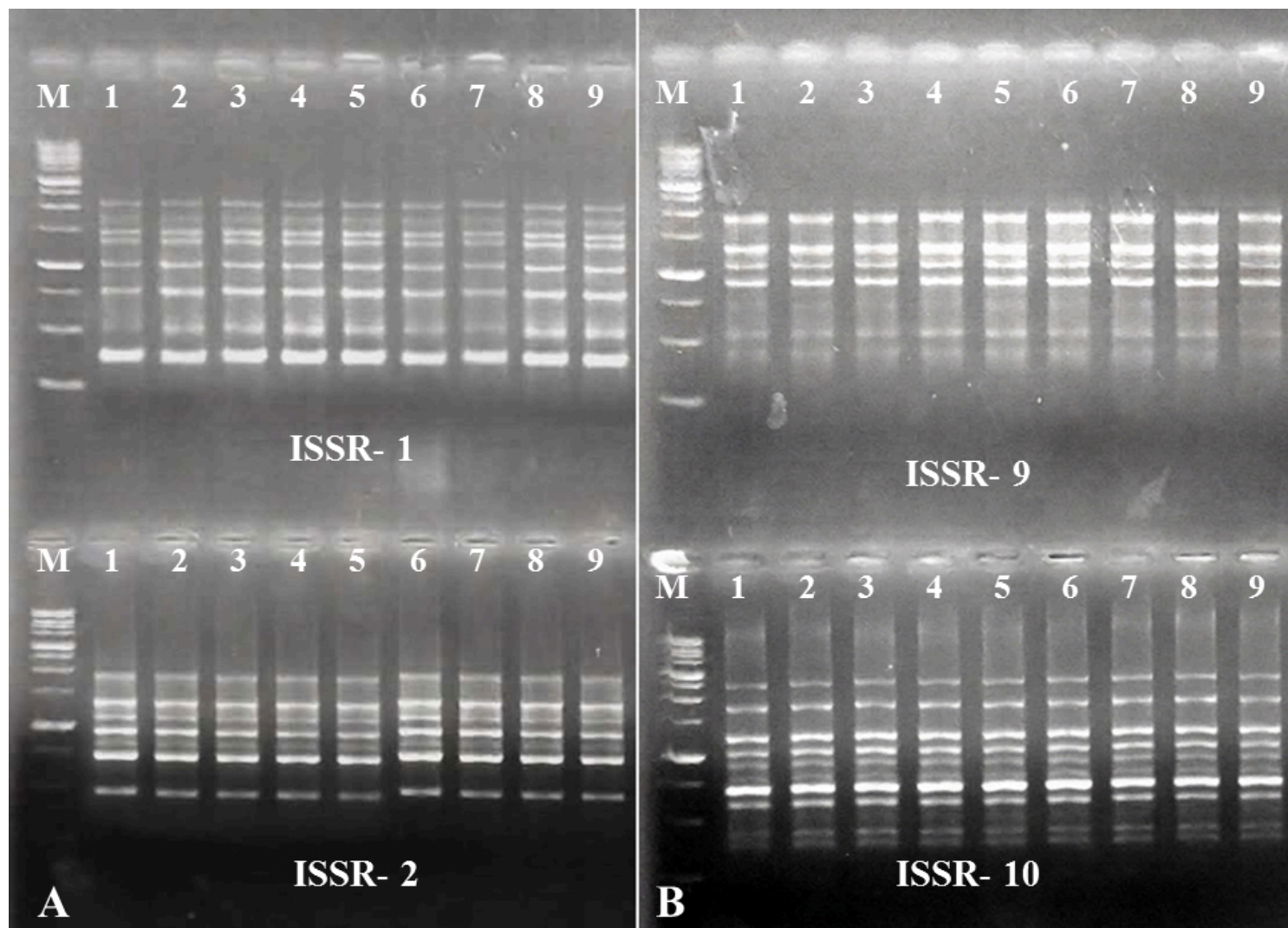


Figure 4

Gel profile for genetic stability for treated samples with several concentrations of CFCF using ISSR-PCR technique A) ISSR-1 and ISSR-2, and B) ISSR-9 and ISSR-10 M: marker (1kb), 1: Control, 2: Filtrate (15%), 3: Filtrate (20%), 4: Filtrate (25%), 5: Filtrate (30%), 6: Autoclaved (15%), 7: Autoclaved (20%), 8: Autoclaved (25%), and 9: Autoclaved (30%).