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Data Mining Software Development
International Conference

The 1st International Conference of Data Mining and Software Development (DSD 2020)

ICBE

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Welcome massage from SRO

We would like to welcome you to our conferences. It is our pleasure to have you with us and being with you. Due to the international COVID-19 prndemic the conference is being held this year as an online event at Stockholm, Sweden on 26 June 2020.

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These conferencse are one of our activities which aim to connect between scientists, researchers, acedimics and industrial experts from different countries to share their views and discuss their advanced research work in the various topics of Biotechnology, Environment and Engineering Sciences. The conferenes are an excellent platform for academic exchange and cooperation promotion. It provides an excellent opportunity for researchers, scientists and postgraduate students to interact and build up academic relationship.

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Abstracts

Assessment the Effects of Land Use / Land Cover changes on soil loss and sediment yield using WaTEM/SEDEM model: Case study of Ziz upper watershed in SE-Morocco

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Abstract

Water erosion and sediment production rates are closely related to land use and land cover (LULC). A spatially distributed soil erosion and sediment delivery model can be used as a good tool to assess the effects of changes in land use on erosion processes. However, their calibration requires a lot of data, sometimes non-existent. In this work, WaTEM / SEDEM model of spatial distribution of sediments and their delivery to rivers was applied to the large Ziz basin (4,435 km^2) in south-eastern Morocco. Model calibration was carried out based on recorded sediment yield during the period from 1973 to 1990 at Hassan Eddakhil dam in the catchment outlet. Validation of the model results was made with recorded sediment yield from 1991 to 2009. Thereafter, three LULC scenarios were modeled by reproducing land use / land cover in 1936 and in 1957 and then a hypothetical future scenario. The comparison of these simulations with the current situation of LULC shows that sediment yield increases from 1,5 Mt. yr-1 to 2,2 Mt. yr-1 and an increase in specific sediment yield from 0.37 t. ha-1. yr-1 to 5.6 t. ha-1. yr-1. The results revealed that forest land drastically declined while wetlands substantially increased between 1936 and 2017.These results can be explained by the interactions between bioclimatic factors and ecologically inadequate and destabilizing human interventions (overgrazing, deforestation). The great anthropogenic pressure on the natural resources which lasted in time ended by the outcropping of pavements of soils exposed directly to the erosive processes.

***Keywords*:** LULC change, WaTEM/SEDEM, GIS, Sediment yield, Ziz watershed, SE Morocco

The Beni Mellal-Tagleft Geotrail (Central High Atlas of Morocoo): A geotourism and educational potential to develop and to preserve

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Abstract

The Béni Mellal-Khénifra region has an important geological heritage both by its richness and diversity. It is characterized by a set of sedimentary, stratigraphic, magmatic, paleontological and karst geosites of significant scientific, pedagogic and aesthetic nature.

The objective of this study is on the first hand to carry out an inventory of potential geosites existing along the Beni Mellal-Taguelft geotrail which constitutes a port at the M'goun geopark, and on the other hand, to develop this heritage through the development of a hiking circuit to discover this rich heritage. In this communication, we will present the karst geosites of the Beni Mellal Dir, the magmatic geosites of Idemrane, J.Sgat and Ait Boulmane located east-south of the Beni-Mellal city.

The karst geosites of Jbel Rat formation constitute one of the most spectacular geomorphosites which fascinate both foreign and Moroccan visitors; these are the limestones and dolomites of the Lias located 15 km east-south of the Beni Mellal city, affected by the surface and underground karstification processes linked to the dissolution of carbonates. This dissolution leads to the formation of landscapes of great scientific and aesthetic value (cliff, lapiaz, sinkholes, poljés, downpours, caves, ruiniform reliefs, resurgences ...) which are distinguished by their great attraction degrees of tourists and their pedagogical importance that testifies to the hydrogeological functioning of Mediterranean karsts.

The Idemarne geosite, located 20 km from Béni- Mellal and south of J.Taçemet, contains sills which have thickness about 10 to 40 m and extending more than 10 km and a magmatic intrusion within the marlcarbonate formation of Bin El Ouidane (of Middle Jurassic age). In addition, it offers beautiful panoramic views of Béni- Mellal city and the Tagleft syncline. This is an aesthetic site especially during the winter season when the mountains peaks are capped with snow.

As for the magmatic geosite of J.Sgat, located 6km south of Idemrane geosite, it represents another mode of magmatic deposit: it is two basalt flows of tabular structure. These flows cover J.Sgat and are presented in prismatic form. The flows are cut by deep valleys and drinking water sources. In addition to its magmatic and sedimentological interest, this geosite

offers the visitors exceptional panoramic views of the Tagleft syncline, Oued El Abid and the yellowish intrusion of Idemrane.

The Taghya Ait Boulmane geosite is a very special site; it contains a very diversified geo-heritage: a magmatic gabbroic intrusion accompanied by enclaves of Triassic pink clay. This intrusion occupies the heart of a calcareous anticlinal wrinkle of Sinemurian-Carixian age attached to numerous radiating dykes; the latter penetrate red layers of the Guettioua formation. The contact between the magmatic intrusion and the limestones flint is underlined by faults. These limestones are very rich in fossils represented by gastropods, lamellibranches and cephalopods (ammonites) and constitute a very developed karst landscape (lapiaz, ruiniform reliefs and caves). Also, the study area has significant cultural and historical potential marked by the presence of a very interesting architectural heritage, in the form of agglomerations of community granaries built of clay and stones.

These important pedagogic, scientific and aesthetic geosites have become in recent years attractive areas for tourists, especially foreigners. However, the anarchic and massive exploitation practices of these sites, in order to extract building materials, seriously threaten the preservation of this geological heritage and the panoramic views which can participate in the socio-economic development of the region.

Keywords: Heritage, Geotourism, Inventory, Geotrail, Promotion, Beni Mellal Atlas, Jurassic-Cretaceo

Quality control for herbal medicinal plants using a sensor array (an electronic tongue)

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Abstract

Herbal medicinal plants have a crucial role in health and social concepts worldwide. The quality of these products affects the expected remedies when using them. There are several analytical methods used for identifying the composition and following these products shelf life. The objective of this study is to use an electronic tongue (ET) for quality control of a local herbal medicinal plant product called Relax. Two multivariate data analysis (MVDA) techniques, namely principal component analysis (PCA) and hierarchical cluster analysis (HCA), were used to analyze the signals of measured samples. ET was able to follow the shelf life of the product and could identify when there was a change in its ingredients and this help in the benchmarking of the product. The obtained results may suggest the use of ET as a good tool for herbal medicinal plant quality in the future.

***Keywords*:** electronic tongue (ET), herbal medicinal plants, PCA, HCA, benchmarking.

Impact of Wind Speed and Direction on Low Cloud Cover over Baghdad City

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Abstract

Clouds are one of the best evidence for the continuous movement of the Earth's atmosphere if they play a major role in the Earth's climate through their influence on the balance of solar radiation .If it absorbs part of the falling solar radiation, and this causes the heating of the Earth’s surface. Clouds also work to reverse part of the falling solar radiation, and then it will cool the Earth’s surface. The methods used in the study depend on the daily, monthly, and seasonal mean of Low Cloud Cover (LCC), Wind Speed (WS), and Wind Direction (WD) are taken from the European Center for Mediterranean Weather Forecast (ECMWF) for the year 2018 at the time hours (00.00 am, and 12.00 pm) over Baghdad Station. The largest value of low cloud cover was recorded during December, January and February, while wind speed was low, the highest value of wind speed was during June and July at 12:00 pm. As for the seasonal analysis, it was noted that the LCC is high during winter and autumn. The correlation strength between wind speed and low cloud cover was also found to be inverse where the higher the wind speed, the less low cloud cover.

Keywords: Low cloud cover, Wind speed, Wind direction, ECMWF, Baghdad.

A Numerical computation of airflow over Iraq regions

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Abstract

The best way to understand the general atmosphere system is to collect and analyze data, identify the variables that occur in the upper and lower classes, and compare them with other values in favor of comparing them to other studies and research. Studies have been conducted in this research by analyzing the wind speed and direction and comparing it with the surface roughness to reach a concept by dividing the regions of Iraq on the basis of the surface roughness that affects the wind speed near the surface of the earth. The methods used in the study depend on the hourly rates of surface roughness, wind speed and direction taken from the European-Mediterranean Weather Forecast (ECMWF) for a full year (2016(For 34 stations over Iraq. Results obtained from wind speed analysis and trend data. The highest value of wind speed (6.5 m / s) in the less rough areas (0-50 m) is concentrated in the semi-desert in the southern and western regions of the country (Anbar, Najaf and Smawa) and the lowest wind speed (1.8 m / s) for the rough areas (11- 72 m) in the mountainous regions in the northern part of the state in the governorate (Erbil, Zakho, Sulaimaniyah).

Keywords: climate change, wind speed, wind direction, surface roughness, airflow.

Effect of Putrescine and the Lighting type on *Gardenia jasminoides* L. callus content of some active medical compounds

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Abstract

The role and importance of medicinal and aromatic plants is due to the antioxidant properties of its components, usually associated to a wide range of amphipathic molecules, broadly termed Phenolic compounds. Therefore, the research aims to increase the production of active medicinal compounds through the treatment of callus *Gardenia jasminoides* L plantlets with two different types of lighting and different concentrations of polyamine, putrescine. The experiment was conducted in the tissue culture laboratory belonging to the department of plant production techniques, AL-Musaib technical college, the experiment included two factors, the first included two sources of Lighting type, the regular light (fluorescent) and LED light (18 red: 2 blue), and the second factor adding putrescine in three concentrations (0.5, 1, 1.5) $mg.L^{-1}$ in addition to the control concentration, Some phenolic substances (Coumaric, Ferulic, Caffeorylquanic, Sinapic acid, Tannic acid) were estimated in *Gardenia jasminoides* L callus using the high-performance liquid chromatography technique Hplc. The data were analyzed using the Genstat statistical program, and the averages were compared according to the LSD test at 0.05. The results showed that the LED lighting treatment was significantly excelled in the concentration of all the measured compounds, and the putrescine treatment at a concentration of 0.5 $mg.L^{-1}$ gave the highest concentration of the compounds (Ferulic, Caffeory lquanic, Sinapic), while the concentration 1 $mg.L^{-1}$ gave the highest concentration of the compounds (Coumaric, Tannic acid). Also, the Bi-interaction (LED + Putrescine at concentration 0.5 $mg.L^{-1}$) gave the highest concentration of compounds (Ferulic, Caffeory lquanic, Sinapic) in *Gardenia jasminoides* L callus.

Keywords: *Gardenia jasminoides* L, HPLC, Phenolic Compounds

Mycodiesel production from oleaginous fungi isolated from oil-rich soils in Basrah

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Abstrac

The present study was aimed to determination of mycodiesel production from oleaginous fungi that included *Aspergillus niger*, *A.flavus*, *A.ochraceus* , and *Penicillium chrysogenum*. Three different fermentation media were used to cultivated fungal isolates that included nitrogen limiting medium NLM, chees why medium CWM, and waste molasses dates medium WMDM, the accumulation lipids of drying biomass were extracted by using 1:2 methanol: chloroform . The result of biomass and lipids production were showed that NLM and CWM represented the best media for accumulation of lipids and yield high biomass, while *A. niger* and *P.chrysogenum* were accumulated high percentage of lipids by 36% and 38% respectively. Furthermore, the analysis of fatty acid methyl ester FAMEs was used by transesterification reactions of extracted lipids. Subsequently, the FAMEs were analyzed by using GC/MS then Cetane Number (CN) of mycodiesel was calculated, therefore, the results were showed that the percentage of the composition of FAME ranged between 66.06 - 92.29 % , while the results of CN was 52.6 - 57. On the other hand the results of residual carbon and sulfur content of mycodiesel product were 0.04 and 0.0069 wt% respectively.

Isolation of *Bacillus* genus from different cave ecosystems; an Algerian case study

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Abstract

Cave microbiology is a growing interdisciplinary field involving efforts of microbiologists, geologists, and chemists. However, this discipline is poorly studied in Algeria. Moreover, probiotics isolation from other origins than the human origin is insufficiently investigated. The aim of this study is to isolate bacteria of the genus *Bacillus* from caves in order to study their probiotic and enzymatic potential.

A number of 16 samples from 8 different cave types (Carbonate caves, sandstone, horizontal, vertical, with presence and absence of bats) from different cities in Algeria (Constantine, Guelma, Bejaia, Tebessa, Bouira) had been studied.

Bacillus isolates had been selected by using heat treatment method. Isolates safety studied using blood agar test, lecithinase test and *Bacillus cereus* medium test. Ability to degrade gliadin was tested by using gliadin as a sole nitrogen source in solid medium and liquid medium. Probiotic potentialities had been also studied (growth in 37°C, antibiotic resistance and resistance to different pH range). In the other hand, physicochemical, soil, water and organic matter parameters had been measured.

Results showed 15 isolates of *Bacillus* (selected from a number of 250 isolates) with non pathogenic character, ability to degrade gliadin in solid and liquid medium and a probiotic profile. Our study confirmed the ability of cave microorganisms to rapidly reproduce at oligotrophic conditions which provide it an industrial importance. In addition to that, the enzymatic potentiality of isolated *Bacillus* to degrade gliadin (the primary cause of celiac disease) accords it the possibility to be used as nutrient supplementation for patients with gluten intolerance (celiac disease). Furthermore, the ability of resistance to different pH ranges and temperature confer it the capacity of being used as a probiotic supplementation. Finally, research of probiotics in other ecosystem than human origin may lead to discover better resources to digestive diseases.

Further tests considering the probiotic potentiality and the safety of selected *Bacillus* are required .As well; a molecular identification of selected microorganisms is needed.

***Keywords*:** Caves, *Bacillus*, extracellular enzymes, celiac disease, gliadin.

Inhibition tests and study of metabolites in fungi that deteriorate stony materials

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Abstract

A group of different fungi isolated from many kinds of Spanish monuments was used in present study. This work focused on three main objectives:

A) Metabolism of fungi harboring the stones by means of HPLC.
B) Study effect of four biocides, i.e. Actidide, Meragel, Preventol, and Tego on fungal growth and sporulation.
C) Electronic microscopy examination of the growth of those fungi on the stones.

The results showed that some phenolic acids, i.e. Gallic acid, Caffeic Acid, Gentisic acid, Vanillic acid, Coumaric acid, Ferulic acid, 4hidroxy benzoic acid, and 4hidroxy-phenyl propionic acid, were produced. Electronic microscopy examination showed inhibition of the fungi growth and changes of their morphology and structure. Also, results postulated that Actidide was the best biocide for controlling growth of the tested fungi at concentration of 0.001%.

Key words: Fungi, Biocides, Biodeterioration, phenolic acids, Actidide.

Full Papers

Quality control for herbal medicinal plants using a sensor array (an electronic tongue)

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Abstract

Herbal medicinal plants have a crucial role in health and social concepts worldwide. The quality of these products affects the expected remedies when using them. There are several analytical methods used for identifying the composition and following these products shelf life. The objective of this study is to use an electronic tongue (ET) for quality control of a local herbal medicinal plant product called Relax. Two multivariate data analysis (MVDA) techniques, namely principal component analysis (PCA) and hierarchical cluster analysis (HCA), were used to analyze the signals of measured samples. ET was able to follow the shelf life of the product and could identify when there was a change in its ingredients and this help in the benchmarking of the product. The obtained results may suggest the use of ET as a useful tool for herbal medicinal plant quality in the future.

***Keywords*:** electronic tongue (ET), herbal medicinal plants, PCA, HCA, benchmarking.

1. Introduction:

Herbal medicinal plants play an essential role in the treatment of several diseases throughout history. There is recently an international interest in the issue of quality of these types of products. Since the quality will influence the effect of these plants on the human, the research has focused on the active ingredients and quality of herbal medicinal plants related to antimicrobial resistance and antioxidant activity effects [1].

For the antimicrobial resistance research, Silva *et al.* [2] tested the antimicrobial resistance against many herbal plants. The results showed potential antibiotic inhibition effect of the pomegranate (*Punica granatum*) fruit extract against twenty-two of the thirty-eight *Staphylococcus aureus* strains being tested. Moreover, a noticeable antimicrobial effect of ginger (*Zingiber officinalis*) oil against *Staphylococcus aureus*, while *Escherichia coli* was greatly affected by both clove (*Caryophyllus aromaticus L.)* and *Cinnamomum zeilanicum* Blume oils [2].

At the same time, inhibition effect was observed toward one of the tested bacteria including *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae, Morganella morganii* and *Pseudomonas aeruginosa.* The antibacterial effect was due to at least using eleven plant species originated in Argentine [3]. The phenolic compounds presented in chamomile were found to have antimicrobial activity against *Staphylococcus aureus* [4].

Regarding the antioxidants activity, a group of aromatic plants in Greece were tested for their oxidation capacity, total phenolic contents, reducing power and stability through oxidation and showed consistent results [5].

Sabbobeh *et al.* [6] studied both antimicrobial resistance and antioxidant activity for the popular Palestinian plant *Salvia palaestina* (Lamiaceae) leaves. A disc diffusion method was used to estimate the antimicrobial activity of essential oils against two bacterial strains *Staphylococcus aureus* and *Staphylococcus coli* compared to gentamicin. The effect of essential oils on *aureus* was greater than that of gentamicin, while the essential oils and gentamicin had nearly the same activity on *Escherichia coli*. Moreover, there was an increase in the antioxidant activity with time and with increasing concentration of the essential oils.

Several types of equipment can be used for the quality control of herbal medicinal plants, *e.g.* gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and liquid chromatography-mass (LC-M) [7, 8]. These techniques have long analytical success. On the other hand, they also have some limitations; including long analytical duration, high-cost analysis, complex sample preparations and the need to have a skilled person [9].

An electronic tongue (ET), which is a chemical sensor, can be a good alternative for analyzing the quality of herbal medicinal plants [10]. ET is composed of an array of chemical sensors with nonspecific, low selective, high stability and cross-sensitivity to different species in solution. Multivariate data analysis (MVDA) can be used for identification and quantification of analyzed samples. There are several techniques for MVDA, *e.g.* principal component analysis (PCA) [10,11], hierarchical cluster analysis (HCA) [12], discriminant function analysis (DFA) [13] and artificial neural network (ANN) [14].

ET has received increased attention across several domains compared to other analytical methods. Since it is fast with rapid sample preparation, reliability, useful in identifying and quantifying of several liquid compounds, acceptable accuracy and relatively low cost [15]. It has been used in many applications, *e.g.* biotechnology, agriculture and environment [16-28]. Moreover, ET was used for quality assessment of some taste chemicals that influence the tea [29] and herbal medicinal plants mixtures quality [30].

This work aims to investigate the possibility of using ET, as fast and reliable quality control equipment, to study the quality and stability (*i.e.* benchmarking) of a local herbal medical product called Relax, during one year of production. This research can open a wide window for using ET for further quality control applications in agricultural and biological sectors to serve the community.

2. Materials and Methods

2.1. Relax herbal medicinal product

The Relax granules product is produced by a local company called Bajjora Company, Tulkarm, Palestine. The product is used as food complement to improve gastrointestinal tract functions (*i.e.* herbal mixture used as a laxative), and it could influence positively in antimicrobial and antioxidant resistance. Moreover, it can be used in weight loss programs and lower cholesterol as well as harmful lipids levels. The ingredients of the product are oats, wheat germs, flax seeds, fennel, mint, caraway, anise, coriander and mahaleb cherry (*Prunus mahaleb* L.).

2.2. Benchmarking of Relax granules

Twelve different samples were collected randomly in December month (*i.e.* end of 2017) from Bajjora Company in Tulkarm, Palestine. Taking into consideration that each sample was produced in a different batch during the year, and there was one batch of production for this product each month (*i.e.* the samples covered one year of production). The manufacturing company had changed in the composition of the product, since they stopped using one of the herbs ingredients in the mixture (*i.e.* mahaleb cherry) from the end of February month. Table 1 shows the production date of each sample.

Table 1. Samples of Relax granules batches and their production month /2017.

Sample Nr.	Production month
1	January
2	February*
3	March
4	April
5	May
6	June
7	July
8	August
9	September
10	October
11	November
12	December

*: The manufacturing company had stopped adding mahaleb cherry to the ingredients after the end of February.

2.3. Sample preparation

Samples for herbal product testing were prepared according to previous studies [31]. For each sample, nine grams (*i.e.* one teaspoonful) of the product were weighted and mixed with 200 ml of boiled deionized water, infused for about 10 minutes and filtered by white gauze pads. Then the resulted solution was cooled to 25℃ (room temperature) before being submitted to ET analysis.

2.4. Electronic tongue (ET)

Potentiometric electronic tongue produced by Astree II (Alpha MOS, Toulouse, France) was used. The device consists of four parts including liquid auto-sampler with 16 bakers, a sensory array, a data acquisition electronic unit and advanced software for analysis (Figure 1).

Sensors array is composed of seven solid potential sensors that are chemically modified field-effect transistors (ChemFET) and Ag/AgCl reference electrode. These sensors consist of two parts: the sensitive layer and the transducer, and are coated with a specific membrane (chemical compounds) to induce both cross-sensitivity and cross-selectivity. Product samples were measured by ET according to the procedure from Alpha MOS. The average of three measurements of each sample was considered for the data analysis [32], which was carried out using PCA and HACA. Both methods are unsupervised classification methods and were used successfully in many applications [33].

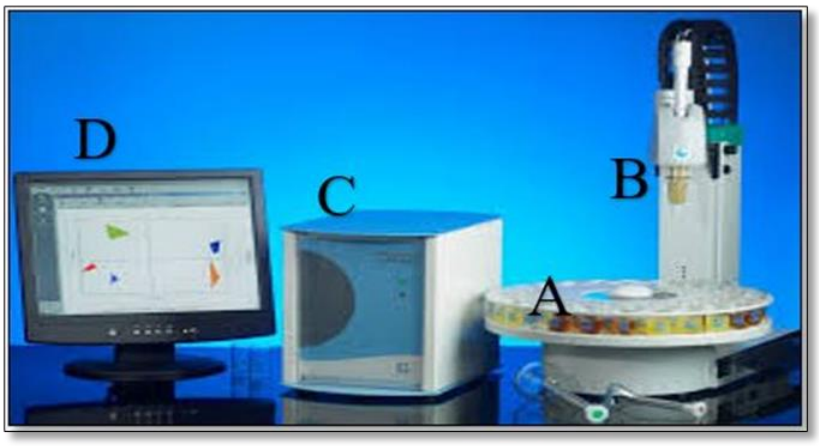

Figure 1. Alpha MOS Astree II parts: (A) liquid autosampler with a capacity for 16 bakers, (B) electrochemical sensor array, (C) acquisition unit, (D) Astree Software V12.4 (Alpha MOS, 2009).

3. *Results and discussion*

Two sensors of ET, namely ZZ and ET, were the most sensitive to Relax batches. The ET signals were stable during measurement samples. The sensors showed good reproducibility, *i.e.* values of standard deviation (SD) (5.6-10.4 mv) were less than 30 mv. Moreover, low values of the coefficient of variation (CV) (*i.e.* 0.31%-0.61%) represented good reliability according to the manufacture of ET [32].

A PCA was carried out. Figure 2 shows the scores plot of PCA. The scores plot explains the relation between samples. Two principal components (PCs) explain 100% of the variation of the data, which indicates that the model can explain the whole variation of the original data, without any significant loss in the information. It can be noticed that there are two main groups in the scores plot. One of the groups is for samples which were taken in January until February. The other group is for samples that were produced in March until December. The classification is most likely due to the difference in the herbal ingredients, since the company had decided to stop adding mahaleb cherry to the mixture ingredients from the end of February month. The ET could identify the difference in the herbal medicinal constituents, even though they usually added mahaleb cherry in a low quantity. This indicates that ET can detect that small difference reflecting the high sensitivity of this taste sensor in predicting the composition of samples.

It can also be noticed that all samples are relatively close to each other, indicating that the storage time (shelf-time) have little or no effect on the analyzed batches ensuring the good quality of this local product. Consequently, the products were kept in a suitable storage condition.

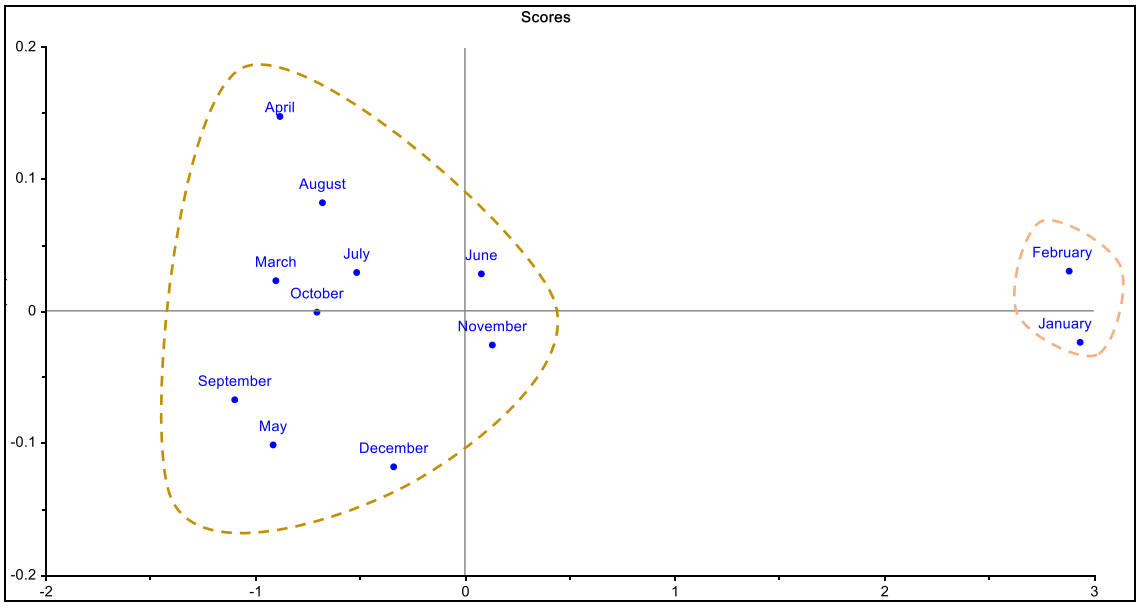

Figure 2. Principal component analysis (PCA) scores plot of the Relax batches samples (collected during months of 2017) measured by ET.

The data of ET was also analyzed using HCA. The dendrogram of HCA classifications is shown in Figure 3. It can be noticed that HCA has mainly two groups (*i.e.* A and B). The first group (*i.e.* A) represents the product in January-February and the second group (*i.e.* B) is for batches of March-December months. It can also be noticed that there are subgroups (*i.e.* B1 and B2). However, the distance between them is small, and this indicates that there is homogeneity in the production and the shelf life has not much effect on the product quality.

The classification (two main groups in HCA) is expected to be due to the same reason that PCA managed to classify the two groups, *i.e.* changing in the product composition, which is due to stop adding mahaleb cherry to the mixture ingredients from the end of February month.

From data analysis viewpoint, it can be that both MVDA techniques managed to get the same interoperation of results.

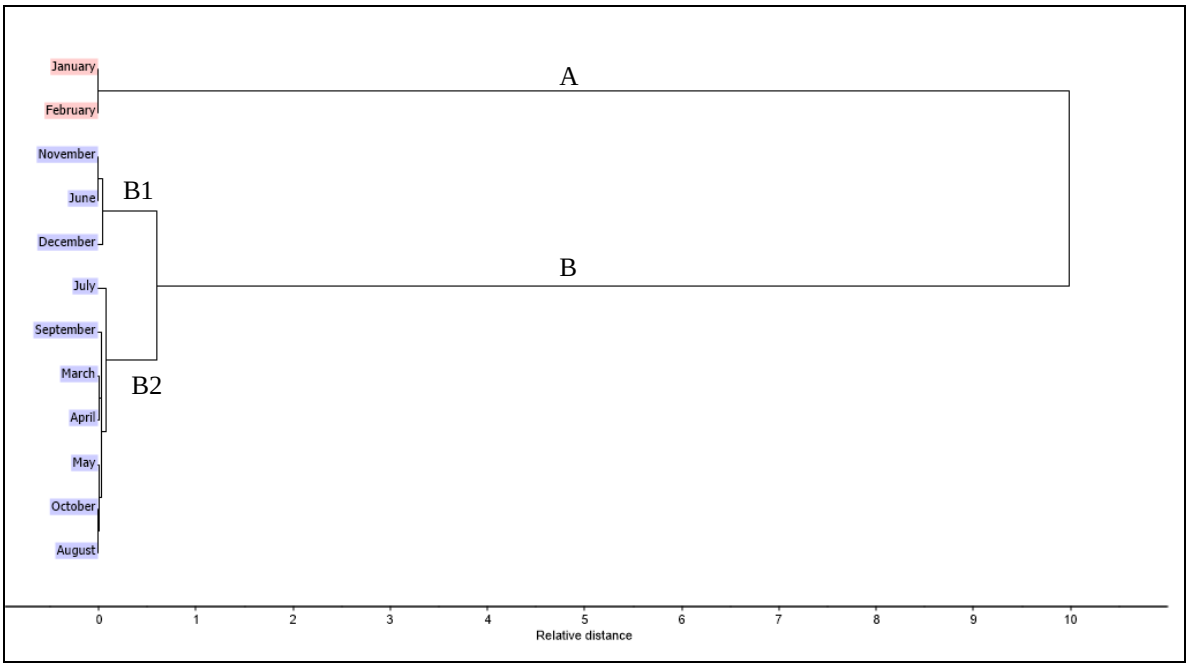

Figure 3. Dendrogram showing clustering of Relax herbal medicinal samples measure by ET. Two groups (A and B) are presented. The group in which mahaleb cherry was added to ingredients (A: in pink colour), and the group in which mahaleb cherry was not added to ingredients (B: in violet colour). Subgroups of B (*i.e.* B1 and B2) have little differences between them, which indicates that the product is homogeneous and there is a little effect of shelf life.

This study produced results which corroborate the findings of a great deal of the previous work in using ET for herbal products, tea [9,29,31], biotechnology and pharmaceutical applications [14,19,20,23,27]. Moreover, the present study extends the knowledge of using ET for different applications.

4. Conclusion

In this investigation, samples of local herbal medicinal plant product called Relax were measured by electronic tongue (ET) to check its quality and benchmarking during one year (*i.e.* 2017) of production. From the results obtained, it can be concluded that:

1) ET was able to follow the herbal medicinal plant storage time (shelf life).

2) The ET signals indicated that there was little effect of the storage time on the homogeneity of the product, and this indicates the proper storage in the manufacturing plant.

3) ET was also successful in predicting the changing in the ingredients of the product (*i.e.* benchmarking), even though the change was in one compound that usually added in a small amount.

4) Both PCA and HCA techniques were able to classify the samples according to the internal characteristics (composition) of the herbal medicinal plant products.

ET showed high efficiency in quality control of Relax product. Further studies are needed to develop innovative analytical methods using ET with other herbal medicinal plants/products and other biotechnology applications.

Acknowledgement

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Inhibition tests and study of metabolites in fungi that deteriorate stony materials

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Abstract

A group of different fungi isolated from many kinds of Spanish monuments was used in present study. This work focused on three main objectives:

D) Metabolism of fungi harboring the stones by means of HPLC.
E) Study effect of four biocides, i.e. Actidide, Meragel, Preventol, and Tego on fungal growth and sporulation.
F) Electronic microscopy examination of the growth of those fungi on the stones.

The results showed that some phenolic acids, i.e. Gallic acid, Caffeic Acid, Gentisic acid, Vanillic acid, Coumaric acid, Ferulic acid, 4hidroxy benzoic acid, and 4hidroxy-phenyl propionic acid, were produced. Electronic microscopy examination showed inhibition of the fungi growth and changes of their morphology and structure. Also, results postulated that Actidide was the best biocide for controlling growth of the tested fungi at concentration of 0.001%.

Key words: Fungi, Biocides, Biodeterioration, phenolic acids, Actidide.

DOI:

1.Introduction

All stony materials exposed to the action of the Nature deteriorate continuously as a result of the action of physical, chemical and biological agents. For several decades many monuments that have in their life over many years, they are suffering a much more intense wear due to undoubtedly, aggressive action of atmospheric pollutants. The problem is more serious since most of the historical buildings are located in urban areas and close to industrial centers. In these areas the continuous emission of pollutants from explosion engines and factories, causes the expulsion of gaseous compounds and particles of different nature, SO2, CO2, N oxides, etc., which are adhere to the surface of the stones, being able interact with it (1). This causes several problems. On the one hand, the prolonged adhesion of particles on the monuments it creates

blackish skates that damage the value aesthetic of the monument. On the other hand, some pollutants deposited, in the presence of moisture, produce compounds highly reactive (H2SO4, HNO3, etc.) that damage the structure of the stone (2). Although, generally, atmospheric pollutants are harmful to organisms, sometimes they can promote microbe's growth. Regarding this, it has been proven that kerosene, one product obtained from oil and consisting of a mixture of hydrocarbons, added to the culture medium, was tabulated and used as a source of Carbone and energy for chemoorganotrophic bacteria (3). Biological agents have long been known (algae, lichens, fungi, bacteria and even higher plants) have an extensive community capable of carrying out chemical and mineral modifications in the different types of rocks and minerals (4,5, and 6). They not only can live on the surface of stony materials but that through the cracks and fissures that often present the walls or the cracks that some organisms penetrate inside and continue their developing and metabolic activity (7). The incidence of microorganisms on materials stony is variable and depends on your metabolic abilities and also, to a large extent, of climatic factors and environmental. Therefore, the study and understanding of the role of microorganisms, and of course fungi, is complex and the numerous interrelationships between the microbial populations and the physical and chemical factors of ambient.

The main source of stone contamination is the soil and through different mechanisms air, humidity, insects, etc., many of these organisms settle and multiply on the stone. Fungi are heterotrophic organisms adapted to live in stone and living together with autotrophic organisms and heterotrophs, forming _ associations, in many houses, synergistic. In addition, yes, due to the hyphae system that develops and the ability to metabolize many polyacids, can be present in fissures, cracks and other cavities of the stone. Fungi have different mechanisms of biodeterioration that can be grouped into two categories: physical and chemical. Due to the physical activity of the hyphae and the pressure exerted during growth can produce fracturing and breaking of minerals and stone. Chemical processes involve deterioration caused by metabolic products. These processes are developed mainly in two ways: through the production of organic and inorganic acids that acidify the media thus favoring the processes of dissolution of the cations minerals. fungi have been found to produce acidity carbonic, nitric, etc. and many other organic acids during their metabolism, such as citric, oxalic, fumaric, gluconic, etc. (8 , 9, and 10). This leads to the formation of salts. organic oxalate, citrate, gluconate, etc., which precipitate and it was deposited on the surface of the minerals. Other Mechanism of alteration is the complexes of chelation that 'for example' many organic acids form excreted by fungi with trivalent cations, thus keeping them in solution (11). A group of different fungi isolated from many kinds of Spanish monuments was used in present study. This work focused on three main objectives: 1-Metabolism of fungi harbored in the stones by means of HPLC. 2-Study effect of four biocides, i.e. Actidide, Meragel, Preventol, and Tego on fungal growth and sporulation. 3-Electronic microscopy examination of the growth of those fungi on the stones.

2.Materials and Methods

2.1 Culture media

The microorganisms used were kept in the laboratory in tubes with inclined malt extract agar, periodically replanting on the same medium and incubating in an oven at 28 "for 7 days to achieve good growth and sporulation. For the conservation of the cultures, paraffin oil that was

poured onto the culture of the fungi grown on malt agar to cover the upper end of the agar. The culture medium used was the Czapek-Dox medium (12)

The glucose solutions are distributed in 100 ml flasks, also containing 10 ml. Sterilization is carried out by flowing steam for 30 minutes, in 3 consecutive days, to avoid the decomposition of glucose. Finally, and under sterile conditions, the saline solution and the glucose solution are mixed in the convenient proportions, to obtain the desired concentration.

2.2Microorganisms

Organisms isolated from crusts and altered surfaces from the cathedrals of Salamanca and Toledo that belong to the culture collection of the C.I.B. The tested bongos are grouped into the following species and genera:

-Aspergillus niger

-*Fusarium oxysporum*

-*Penicillium frequentans*

-*Penicillium steckii*

-*Trichoderma pseudo koningii Rifai*

-*Trichoderma viride*

-*Alternaria alternata*

-*Phoma glomerata*

-*Phoma eupyrena*

-*Mucor hiemalis*

2.3. Reagents

The reagents used in these experiments were the majority of "analytical grade", with the exception of the mineral salts used in the preparation of the culture medium, which were of "reactive grade".

2.4.1. Preparation of the inoculum

From the agar-malt fugal cultures, spore suspensions are prepared. To do this, 10 ml of sterile water containing 0.01% Tween 80 was poured into the solid cultures and the surface was scraped with a platinum handle to obtain the desired spore suspension. Then performed a spore count on a camera counting blood cells "Thoma", adjusting the concentration to 10^5-10^6 cells / ml.

2.4.2. Growing conditions

For the cultivation of the fungi, the Czapek Dox medic with 3% glucose was used. The media was divided into 20 ml fractions in 100 ml Erlenmeyer flasks and inoculated with 1 ml of spore suspension from each fungal culture. After 14 days of incubation at 27 ° C and in the dark, the medic was filtered, the volume was completed to 20 ml and the pH was determined on a Crison digital pH meter, mod. 501.

2.4.3. Preparation of samples for high performance liquid chromatography (HPLC).

After the established culture periods, the samples with fungal culture were filtered through Whatman No 1 paper and the pH in the filtered liquids was measured. Subsequently, the cultures were centrifuged at 7,000 r.p.m. for 20 minutes and the supernatant was subjected, again, to a second filtration through a 0.22 pm pore diameter Millipore membrane. Finally, the filters were frozen until use. 5 ml of each sample was concentrated to dryness on a rotary evaporator. 0.2 ml of concentrated HCl and 0.6 ml of ethyl ether were added to the residue. Shake well and after a period of rest two phases separate. The ether phase is collected and again dried on a rotary evaporator. The sediment is dissolved in 100 ul of (Acetic acid: water, 9:98 v / v). Centrifuge 5 minutes at 12,000 r.p.m. centrifuge leaks microfuge and is injected into the chromatograph or 10 or 20 ul, according to the cases.

2.4.4The phenolic acids used as standards were as follows:

Gallic acid

4-hydroxy-benzoic acid

Gentisic acid

Caffeic acid

Vanillic acid

Syringic acid

4-hydroxyphenylpropionic acid

Acid p. coumaric

Ferulic acid

Standard concentration solutions of 1 mg / ml were prepared and 5 and 10 pl of each of them were injected, separately. In addition, a mix of the 9 patterns was prepared, which was also infected and whose chromatographic branches served as a reference for our problems.

2.4.5 Quantitative analysis of phenolic acids.

Perkin-Elmer HPLC chromatography, 2B series with ultraviolet detector was used together with a 250 x 46 mm Ultrasphere ODS reversed phase column. As gradient, 2 gradient solutions were used. Solution A contained acetic: Milly Q water (9:98 v / v) and solution B consisted of acetic: methanol: water (1:15:34 v / v). The fixed outflow was 0.2 ml / min. The infection volume was 10-20 µl and the wavelength used was 220 pm.

2.5 Biocides

Four biocides were selected to test their inhibition capacity against several isolated fungal strains of altered stone monuments. The interest for the study of these products lies in the fact that they have been used, in their majority, in some countries, together with restoration products, such as supporters and protectors. Biocide samples were donated to the laboratory or the following manufacturers or distributors:

Biocides	Biocide Manufacturer or distributor	Active ingredients
Actidide DW	Core salt	Mixture of cyclic derivates
Mergal V 540	Hoechst Iberica	Triazine and Anunonia derivatives
Tego 518	T.H. Goldschmidt, S.A	Mixture of amphoteric biocides
Preventol R 80	Bayer, S.A	Alkyl Dimeth yl Benzil Ammonium chloride

Diluted solutions of each product were prepared in the appropriate solvent, Actidide in ethanol and the rest in water, so that when added to the Petri dishes, the following final concentrations were reached: 0.001, 0.01, 0.05 and 0.1.

2.6. Study of fungal growth under a scanning electron microscope (SEM).

A Hitachi scanning electron microscope was used in present study. Intact sandstone specimens, about 1 x 1 x 0.5 cm, were prepared and washed and sterilized at 100 'C for 3 days. Spore suspensions of the two strains selected for this study were prepared: *Fusarium sp.* and

Aspergillus niger. The suspensions were made in sterile water with 0.01 % % tween, adjusting cells concentration to 10^5 -10^6 spores/ml.

3. Results and Discussion

Phenolic acids together with flavonoids are their components of the humic substances that are formed during the decomposition of organic residues in the soil, for this reason, phenolic compounds of low pH, appear widely in soils (13). Furthermore, they are also products of microbial metabolism (14). For this reason, in this work we have carried out a study to know the phenolic acids produced by acidogenic and non-acidogenic fungi that had been isolated from Spanish historical monuments. Phenolic acids produced in glucose metabolism were determined by liquid chromatography (HPLC).

3.1 Phenolic acids production:

Table (1) shows the phenolic acids that we have identified by liquid chromatography in the metabolic broths of the fungi. The profile of phenolic acids is shown. There it is observed that *Fusarium sp.* was the fungus that produced the largest number of different acids and also, generally, in the greatest quantity. *P. steckii*, *A. alternata* and *P. eupyrena* followed. On the other hand, the glycol was excreted for all strains studied, followed by the ferulic that was produced by 5 fungi. Most of these acids and also different ones were identified by Martin and Haider (1969) [15] in the *Stachybotrys atra*, *S. chartarum* and *Epicoccum nigrum* cultures growing in media where glucose was the main hydrocarbon component. The absence of some phenols in metabolic broths does not mean that they were not initially synthesized, since it has been found that a wide range of aromatic acids, phenolic acids, etc., can be used as a source of C for many microorganisms [16]. Other works have shown a certain mineral activity of phenolic acids. Robert and Razzaghe-Karimi (1975) [17] found that various types of micas were altered when subjected to the action of p-hydroxybenzoic and vanillic. The remaining fungi tested and cited in Table (1) are not acidogenic, however they synthesize phenols, and in greater quantity, in some cases, than acidogenic fungi, such as *Fusarium sp.* Since the microorganisms do not appear, in an ecological niche, as unique species, but in coexistence with other organisms, sometimes forming microbial consortia, it is possible to think that the complexing effect of these non-acidogenic fungi is favored by the proximity of other fungi and other classes of organisms.

Table (1) :Phenolic acids production (mg / L) for different fungi cultivated on Czapek Dox with 3% glucose during 14 days.

Fungi	GAL	CAF	FER	GEN and / or 4HB	VAN	CUM	4HF	Total	Mean ± SE
Alternaria sp	5,0	3,4	0	0,2	0	8,0	0	16,6	3,37±1,2
Aspergillus niger	1,07	0	0	2,8	0	0	0	3.87	0.55±0,4
Fusarium oxysporium	3,5	4,4	0	0,3	0	0	0	8,2	1, 17±0,71
Fusarium sp	29,0	0	8,6	1,46	31,4	13,2	29,0	112,6	16,1±5,1
Penicillium frequentans	8,8	4,4	1,0	0	0	0	0	14,2	2,02±1,3
Penicillium steckii	21,8	2,25	36,0	3,0	0	10,3	0	73.35	10,47±5,1
Phoma glomerata	23,2	0	30,2	2,0	21,8	0	0	77,2	11,02±2,3
Phoma eupyrena	6,6	0	11,0	0	13,5	11,6	0	42,7	6, 1±6,06
Total	98,97	14,45	86,6	9.76	66,7	43 1	29,0		
Mean± SE	12.37±3,7	1,8±0,7	10,82±5,1	1,22±0,4	8,34±4,4	5,38±2,1	3,62±3,6		6,2±3,46

GAL = Gallic acid, CAF = Caffeic acid, GEN = Gentisic acid VAN= Vanillic acid, CUM = Coumeric acid,

FER = Ferulic acid, 4HB = 4 Hidrox-y Benzoic acid, 4HF = 4 Hidroxy-phenyl propionic acid

3.2 Biocides effects on isolated fungi:

Seven isolated fungal strains of scabs on the outer walls of the cathedral of Salamanca were tested for their growth potential when grown in the presence of biocides. The selected strains belong to the following species: *Penici11iurn frequentans Penicillium sp., Aspergillus niger, Trichoderma viride, pseudokoningii, Mucor hiemalis* and *Fusarium reticulatum.* The interest of these microorganisms lies in that they are acidogenic and have shown that in the metabolism of carbohydrates they produce oxalic 'gluconic' citric malic acids, etc. These acids produced by the fungi are responsible for the alteration of the stony materials since they solubilize the mineral cations of the substrate, carry out a selective attack on silicates and feldspars, mainly [18] and are chelation agents of cations [11, 18]. Actidide biocide was the most effective inhibitors for fungi (Figure, 1). Table 2 shows the Minimum Concentration of each biocide which inhibits fungal growth. It is observed that the minimum concentration of inhibition (MCI) of each product varies with the organism tested. Thus, *Mucor hiemalis* is resistant to Actidide, Mergal and Preventol, at concentrations of 0.001 and 0.01%, however the fungus shows a high sensitivity to Tego.

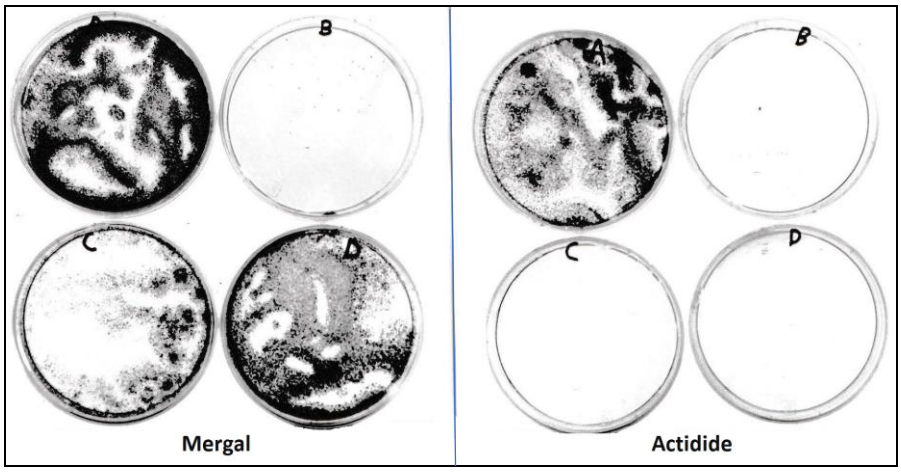


Figure 1: Antifungal activity of Mergal and Actidide against *Aspergillus niger* at different concentrations. A. control, B, C and D, concentrations 0.1, 0.01, 0.001%, respectively.

Table (2): Minimum Concentration of each biocide which inhibits fungal growth.

Species	Minimum Concentration of biocide (%)			
	ACTIDID	MERGAL	PREVEN	TEGO
Penicillium sp	0,001	O,Ql	0,00 1	0,1
Penicillium frequentans	0,001	0,01	0,0 1	0,05
Aspergillus niger	0,00 1	0, 1	0,05	0,001
Tn'chod erma viride	0,001	0,001	0,001	0,05
Trichod enna p seudokoningii	0,001	0,001	0,001	0,001
Mucor hiemalis	0,05	0,05	0,001	0,001
Fusarium reticularum	0,001	0,01	0,001	0,1

T. pseudokoningii, on the other hand, is very sensitive to all biocides tested, in such a way that concentrations of 0.001% are sufficient to prevent the growth of this fungus.

The antifungal potency of each product is reflected in Table 2. There it is observed that under "*in vitro*" conditions, Actidide is the most effective product and, on the contrary, Tego is the product that requires the highest concentration to inhibit microbial growth, especially for *Penicillium* and *F. reticulatum*.

Many factors seem to influence the MIC of a micro-biocide such as the composition of the culture medium, pH, temperature of incubation, inoculum, etc. [19, 20 and 21].

Therefore, after the MIC values of all the fungicides tested were known, 2 strains were selected: *Fusarium sp.* and *Aspergillus niger*, which were inoculated on sand samples and in the presence of Actidide since it was the product that showed the highest inhibitory capacity. Inoculation was performed on stone specimens in order to establish conditions closer to the natural environment and to observe the scanning microscope, the degree of inhibition, and the morphological and structural modifications produced in the fungal cell.

3.3 Electronic microscopy examination of the growth of those fungi on the stones

When *Aspergillus niger* was grown on sandstone specimens with sufficient C and N content, it grew abundantly, forming a biofilm on the specimen surface, as seen under a scanning microscope (Figure 2b). Thin hyphae are well visible across the field and spores form isolated aggregates. However, when *A. niger* was grown in the presence of 0.1% Actidide, concentration at which the fungus was inhibited in Petri dish tests, and added at the same time as the inoculum, growth appears to have been inhibited, the conidia and hyphae, which on the other hand appear altered, (Figure 2a). Then the biocide was applied to the test tube after 7 or 14 days after the growth of the fungus began, the multiplying processes seem to have stopped and the mycelium and conidia observed in Figure 3 would correspond to the cell development produced the days prior to incorporation of the biocide. In addition, there are also crystals, such as the one seen in the lower part of Figure 3 a, probably calcium oxalate, as has been recently verified [22].

When the concentration of the applied biocide was higher, 0.2%, the processes of inhibition followed. With the fungicide added at the same time as the inoculum, it appears that the inhibition is complete (Figure 4 a). When Actidide was added 7 days after the fungal inoculation of the specimens (Figure 4 b), it was possible to observe important changes in the mycelium, such as swellings along the hyphae and also at the tips of the hyphae. In other hyphae, there is vesicular ornamentation on the cell wall, which has also been described in Candida parapsilosis [23] These changes ultimately lead to a progressive destruction of cellular architecture [24 and 25].

Fusarium sp. cultured on sandstone, for 21 days, it shows, at the scanning microscope, abundant growth, developing a branched mycelium that goes through the holes and existing holes in the rock (Figure 5b). Conversely, when *Fusarium sp* was grown in the presence of 0.1% Actidide, important changes must have taken place at the cellular level since growth seems interrupted and the hyphae observed, which must correspond to the inoculum, appear altered, with swellings and

formations similar to those described in *A. niger* (Figure 5a). Figure 6 shows some areas of hyphae affected with very obvious stresses and deformations; in other houses multiform adhesions appear on the hyphae. When *Fusarium sp*. was treated with concentrations of 0.2% of the antifungal agent, the structural changes and the morphological modifications were very marked (Figure 7).

These tests that can be considered intermediate between "*in vitro*" tests and tests on the monument demonstrate the susceptibility of the selected fungal spectra to fungicides as powerful as Actidide.

On the other hand, the biocide added to the indicated concentrations (0.1 and 0.2%) on sandstone samples did not affect the coloration and texture of the stone material, which must be taken into account if what is the aim is to apply it as a micro-biocide on a monument of artistic value.

The next step to be carried out would be the application of 1 biocide limited and controlled surfaces of a monument, laboratory, observing periodically, in tests and the survival of fungal species present on the stone. The concentration of the product it should be variable and small, since in inhibition tests in Petri dishes, Actidide showed ability to inhibit fungal growth at low concentrations such as 0.001%. The efficiency of Actidide due to salicylic acid which reported as one of the most effective agents to control fungal infections [26] (Figure 8).

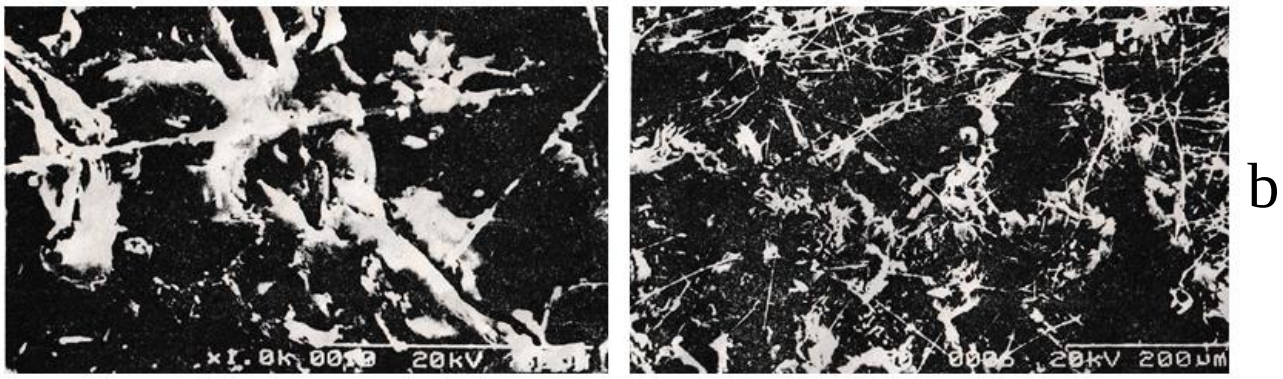
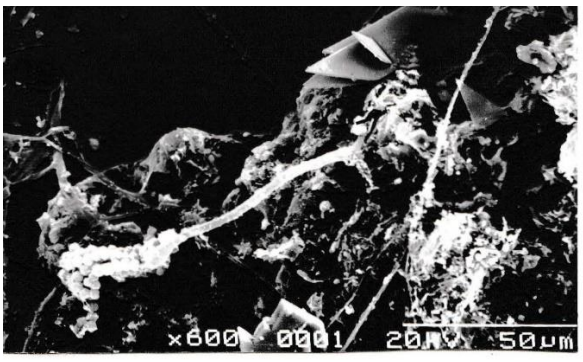
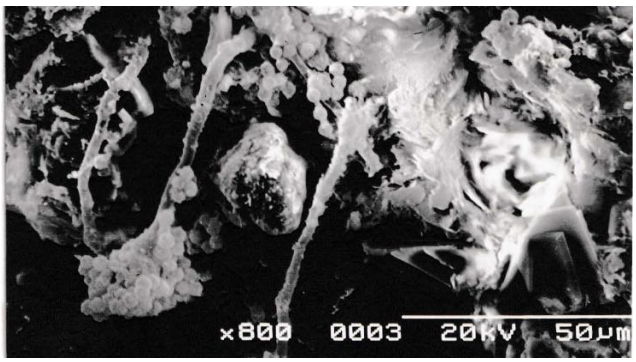
a  b

Figure 2: Micrograph of *Aspergillus niger* growing on sandstone. a) 21 days after treatment with Actidide 0.l% b) 21 days without treatment.

a

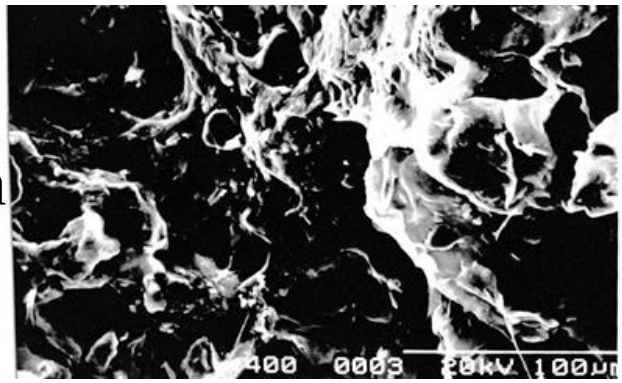


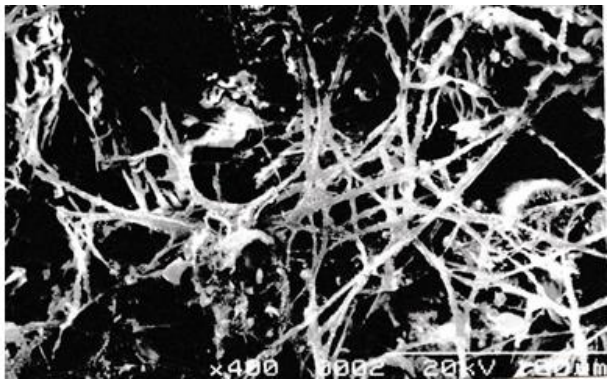


b

Figure 3:. Micrograph of *Aspergillus niger* growing on sandstone. a) 7 days after treatment with Actidide O, l%, b) 14 days after incorporation of the biocide.

a

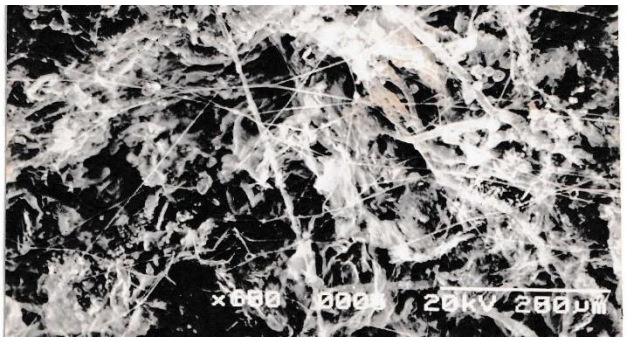


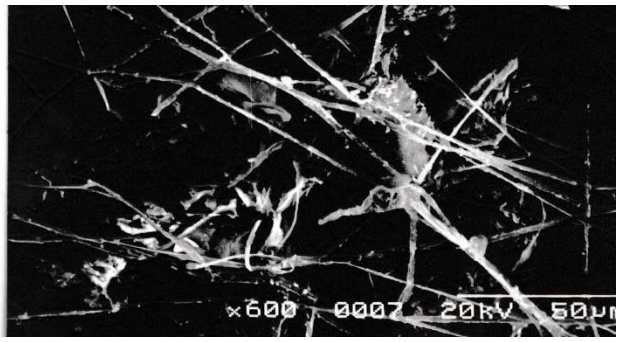


b

Figure 4: Growth of *Aspergillus niger* in the presence of 0.2% Actidide. a) biocide added at the same time as the fungus, b) added 7 days.

a





b

Figure 5: Micrograph of *Fusarium sp.* growing on sandstone. a) 7days after treatment with Actidide 0.l% b) 21 days without treatment.

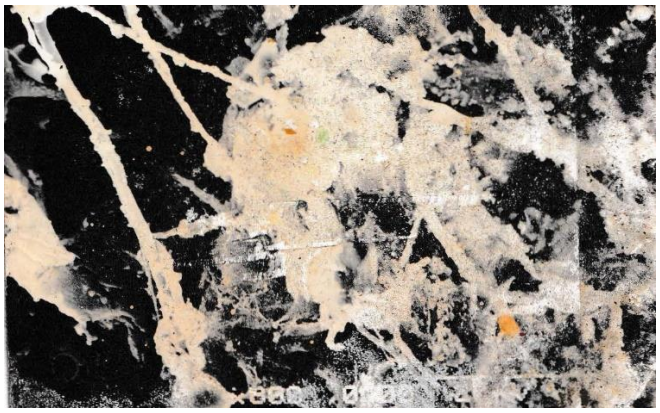


Figure 6: Scanning microscope micrographs of *Fusarium sp*. growing on sandstone after 14 days of treatment with 0.1% Actidide.

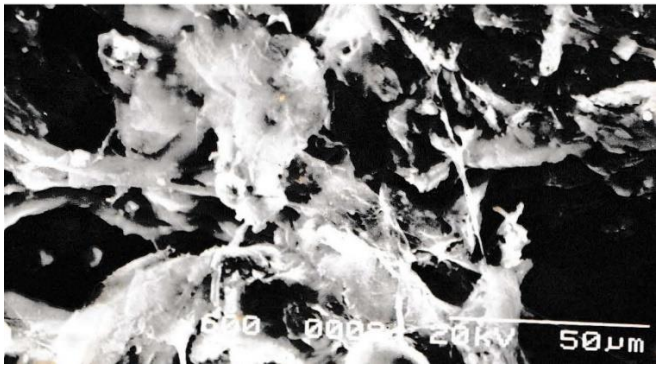


Figure 7: Growth micrograph of *Fusarium sp* in showing the presence of the 0.2% Actidide biocide after 7 days of treatment.

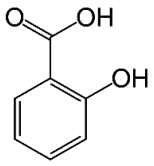


Figure 8: Salicylic acid

4. Conclusion: Actidide is one of the most efficient biocides to inhibit fungal growth at low concentrations for stones treatment. It's efficiency due to salicylic.

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Effect of Putrescine and the Lighting type on *Gardenia jasminoides* L. callus content of some active medical compounds

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Abstract

The role and importance of medicinal and aromatic plants is due to the antioxidant properties of its components, usually associated to a wide range of amphipathic molecules, broadly termed Phenolic compounds. Therefore, the research aims to increase the production of active medicinal compounds through the treatment of callus *Gardenia jasminoides* L plantlets with two different types of lighting and different concentrations of polyamine, and putrescine. The experiment was conducted in the tissue culture laboratory belonging to the department of plant production techniques, AL-Musaib technical college, the experiment included two factors, the first included two sources of Lighting type, the regular light (fluorescent) and LED light (18 red: 2 blue), and the second factor adding putrescine in three concentrations (0.5, 1, 1.5) $mg.L^{-1}$ in addition to the control concentration, Some phenolic substances (Coumaric, Ferulic, Caffeorylquanic, Sinapic acid, Tannic acid) were estimated in *Gardenia jasminoides* L callus using the high-performance liquid chromatography technique Hplc. Data was analyzed using Genstat statistical program, and the averages were compared according to the LSD test at 0.05. The results showed that the LED lighting treatment was significantly excelled in the concentration of all the measured compounds, and the putrescine treatment at a concentration of 0.5 $mg.L^{-1}$ gave the highest concentration of the compounds (Ferulic, Caffeory lquanic, Sinapic), while the concentration 1 $mg.L^{-1}$ gave the highest concentration of the compounds (Coumaric, Tannic acid). Also, the Bi-interaction (LED + Putrescine at concentration 0.5 $mg.L^{-1}$) gave the highest concentration of compounds (Ferulic, Caffeory lquanic, Sinapic) in *Gardenia jasminoides* L callus.

Keywords: *Gardenia jasminoides* L, HPLC, Phenolic Compounds

1) Introduction

Gardenia jasminoides L An evergreen branched woody shrub belonging to the Rubiaceae family, the *Gardenia* genus includes more than 200 species, It is named belonging to the American scientist Alexander Garden (1730 - 1791 AD), China is the original country, the plant height ranges between 1-2 meters and it flowering from mid-May to mid-July to give white flowers with a waxy aromatic smell of bisexual strength and their petals are often composed of several rows (ketmera) [1]. The leaves of the plant are lanceolate to an inverted Ovate, their length reaches about 10 cm, dark green, lustrous, prominent veins confronting in triple

assemblages. It is considered one of the beautiful ornamental shrubs that decorate the home and public gardens, where its flowers are used to extract perfumes as well as use as cut flowers, and it is one of the most important ornamental plants spread abundantly in the world, the main method of reproduction is the cutting, Grafting on strong rootstock resistant to nematodes [2]; [3]. The method of tissue culture in vitro is preferred as it gives a high average of propagation through organogenesis [4]. Gardenia is a Heliophytes and Hygroscopic plant that grows in direct sunlight that is necessary in order to achieve the best flowering production [5]. The tissue culture techniques play an important role in the propagation of many plants, including trees and shrubs, which are difficult to multiply by the usual vegetative methods. Among these plants is the Gardenia. [6] was the first to use tissue culture technology to propagate Gardenia where it succeeded in rooting the recent growth resulting in culture tubes by 75%, To get rid of this low product to multiply this shrub in the traditional methods. The addition of industrial growth hormones to the nutritional media is considered one of the basic and important matters in order to stimulate the plant parts to grow, develop and root formation. Therefore, tissue culture does not succeed without the use of growth regulators [7]; [8]. The polyamines represented by putrescine (Put) are organic compounds of very low molecular weight that contain two or more active amine groups with multiple functions in physiological processes within the plant and are present in all parts and are among the secondary growth organizations that have been recently introduced in research and studies for their effective generation in Most plant development processes [9]; [10]. Putrescine and its plant formula C4H12N2.2H2O are a kind of polyamine and the primary source for the formation of other types (spermidine and spermine). It is also the least amines molecular weight, which gives it a rapid transition between cell components or plant members, where it was found that it plays an important role in cell division, flowering processes and morphological formation [11]. Numerous studies have indicated a multi-amine role in the formation of adventitious roots, where they play an important role in the stage of root development in many woody plants, including those found by [12]; [13], when multiplying different types of citrus origins *in vitro*. The growth of tissue cultures requires the regulation of many conditions such as light, heat, moisture, and carbon dioxide inside the growth rooms [14]. where little or excessive light impedes plant growth or leads to excessive growth, respectively, The quality of lighting also affects morphological characteristics such as stem length and leaf size [15]. In recent decades, Light Emitting Diode (LED) lamps have been used worldwide in agriculture as a new lighting source because of its advantages, the most important of which are small size, little drainage for electric energy, high efficiency and long operating hours (50 to 100,000 hours) with little heat production. These lamps are used in different colors, including white, red, blue, yellow, green, or a mixture thereof, and each colour has its own characteristic, where the function of the red LED light is to induce chlorophyll to manufacture food by photosynthesis, while blue light affects the morphology of the plant [16]. Through research, [17] found that using a mixture of red and blue LED lights (18 red - 4 blue) gave the highest number of branches and leaves in the tissue culture of Rosa *Kordesii*, compared to white fluorescent light.

2) Materials and methods

The experiment was conducted in the tissue culture laboratory belonging to the department of plant production techniques, AL-Musaib technical college, to study the effect of different concentrations of putrescine and the Lighting type in the content of *Gardenia jasminoides* L. callus from some active medical compounds.

Plants material

Gardenia plants took Ellis cultivar from a good growing mother plant grown in a private nursery and it was taken into consideration that it is free from any insect or disease infection at the age of 3 - 4 years and it was approved as a source for taking the plant parts to be propagated tissue cultured in the laboratory after removing all the open leaves.

Sterilizing the used working tools

All tools used in tissue culture were sterilized for the current study from tweezers, scalpels, Petri dishes and filter paper after wrapping them with aluminum leaves and placed in the conductor for 20 minutes at a temperature of 121 C° pressure 1.04 kg/cm^2. During the culture process, the tools were covered with ethanol with a concentration of 96%, and then exposed to direct fire flame during the culture process. As for the sterilization of hands and workbenches, it was by using ethyl alcohol with a concentration of 70% before and during the culture process. The laminar flow cabinet was also sterilized by spraying its internal walls and floors with ethanol at a concentration of 70% and wiped with blotting paper, and it was filled 30 minutes before its use [12].

Sterilizing plant materials (Explants)

After separating the new growths from the mother's plants, they were washed with regular tap water and sterilized with 70% ethyl alcohol and liquid soap, where parts of the leaf blade that were taken from fully leaves were located directly after the apical meristem with a length of less than 1 cm and a width of 0.5 cm container on the middle vein. Then the parts are cleared by leaving them under tap water for 30 minutes. Then it is immersed in an antioxidant solution to get rid of tissue brown damage, Which consisted of ascorbic acid (150 mg.L^{-1}) and citric acid (100 mg.L^{-1}) for 30 minutes and then transferred to the pentomyl solution (fungicide) for 2-3 minutes, followed by rinsing the explants with distilled and sterile water for 3 Minutes. Surface sterilization was then performed for the selected explants parts after they were transferred to the laminar airflow cabinets in $HgCl_2$ solution at a concentration of 0.1% (w/v) for 5 minutes with adding two drops of Tween 20 diffuser with continuous shaking to remove the air bubbles formed on the final parts. In the end, it was washed with distilled and sterile water three times for 3 minutes each time in order to get rid of any harmful effects of sterilization and to preserve the vitality of the explants parts [18].

Prepare the MS nutritional medium

MS was used as the basic medium in the current study. The medium was prepared in the laboratory from nutrient salts according to the recommended concentrations and for preparing one litre of medium dissolving 6 g (Agar-Agar) in 400 ml of distilled water at 1 degree. Boiling and mixing the ingredients using a Magnetic stirrer on the hot plate with the addition of Macro and Micronutrients, vitamins, sucrose and Myoinositol after dissolving it with distilled water to the nutrient medium each according to the required concentration and then complete the volume to 1 L and distribute it in the 200 ml bakeries with the addition of the growth regulator according to the aim of the experiment conducted. Then the baker's nozzles were covered with heat-resistant aluminum foil and sterilized at 121 C° and pressed 1.04 kg/cm^3 with an autoclave for

20 minutes, then the tubes were removed from the autoclave and left to cool at room temperature [19].

Prepare the explants

After performing superficial sterilization of the explant, they were cut into smaller parts (the length of the nodes is about 1.2 - 1.5 cm), where they were transferred to pre-sterilized Petri dishes using pointed end tweezers with sharp surgical blades and thus ready for transplantation [4].

The stage of initiation and multiplication

The first stage 4 weeks after the culture is considered initiation stage, where the leaf blade parts were culture after the sterilization process was completed in test tubes containing 10 ml of pre-prepared solid MS medium on the response of the leaf blade parts containing the middle vein to the callus initiation and its differentiation. The cultures were incubated in the growth room at a temperature of 25 ± 2 C° and the light intensity 1000 lux with 16 hours of light followed by 8 hours of darkness. And then these explants were re-culture for an additional four weeks in the same medium of the initiation stage and under the same conditions and it was considered a multiplication stage [18].

Callus Cultures initiation

When the callus volume reached the appropriate size, i.e., after 8 weeks of culture, it was transferred to a new culture medium after removing the callus mass from the tubes by sterile forceps and placed inside a sterile petri dish and the callus cut into small pieces, For the purpose of re-culture with the aim of studying the effect of the presence of putrescine in three concentrations (0.5, 1, 1.5) mg.L $^{-1}$ in addition to the control concentration in the culture medium under the influence of two sources of lighting type are the regular light (fluorescent) and LED light (18 red: 2 blue) in order to Determine the best combination to influence the type of active substance [17].

Analysis of Phenols

The main compounds were separated on m FLC (Fast Liquid Chromatographic) on reversed phase 3 µm particle size, (50 x 2.0 mm I.D) C-18DB column, separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by Shimadzu SPD 10A vp, the data were recorded on Shimpack C-R8A integrator (Shimadzu, koyota, Japan). The optimum separation condition as follow: Column: FLC (Fast Liquid Chromatographic) column , 3 µm particle size, (50 x 2.0 mm I.D) C-8DB column, Mobile phase were :acetonitrile : tetrahydrofuran (THF):,0.1 % acetic acid (6 : 3 : 1, V/V) detection : UV set at 254 nm , flow rate 1.2 ml.min-1. temp: 40 C [15].

The sequences of the eluted fatty acids standard were as follow (Table 1), each standard was 25ug.ml-1

Table (1) Retention Time and the area of fatty acids

Seq	Subjects	Retention time minute	Area	Concentration
1	Tannic acid	2.13	123026	25 $mg.l^{-1}$
2	Coumaric	4.40	130370	25 $mg.l^{-1}$
3	Ferulic acid	5.50	117958	25 $mg.l^{-1}$
4	Caffeoylquinic	6.70	85243	25 $mg.l^{-1}$
5	Sinapic acid	7.35	93515	25 $mg.l^{-1}$

Quantitative determinations of fatty acids were done by comparison the peak area of authentic standard with that of sample peaks under the same optimum separation condition, by using the following equation [20]:

$$\text{Concentration of sample } \mu g/ml = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. of standard x dilution Factor}$$

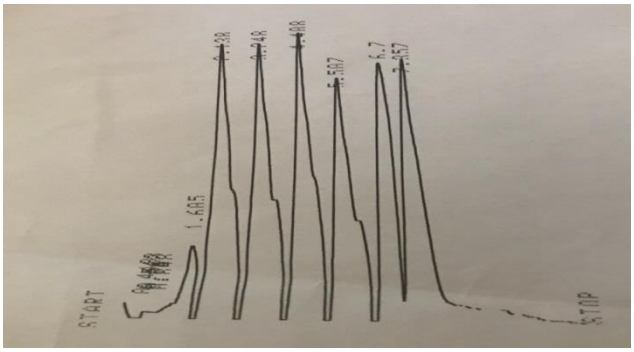

Fig (1) Separation chromatogram of compounds under the optimum separation condition

Experimental design and statistical analysis

A factorial experiment using according to Completely Randomized Design (CRD) [21]. Data was analyzed using the GenStat statistical program, and the averages were compared according to the LSD test at 0.05. Ten replicates were used for each treatment, with one culture vial, for each frequency of multiplication and initiation experiments.

3) Results and discussion

The results of the statistical analysis are presented in Table (2). They indicate a significant difference in the concentration of Coumaric acid in the Gardenia callus, where the LED Lighting treatment gave the highest average of 156.8 µg /mL while the florescent treatment gave the lowest average of 148.0 µg /mL.

Table (2) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Coumaric acid

Lighting Type	Putrescine ($mg.L^{-1}$)				Mean
	0	0.5	1	1.5	
Florescent	122.4	154.2	163.4	151.9	148.0
LED	136.0	160.9	176.9	153.4	156.8
Mean	129.2	157.5	170.2	152.6	
$L.S.D_{(0.05)}$	Lighting 16.01	Putrescine N.s	Lighting *Putrescine 32.01		

The treatment with putrescine significantly affected the concentration of Coumaric acid in *Gardenia jasminoides* L. callus, where it gave the treatment at a concentration of 1 mg/L, the highest average of 170.2 µg /mL, while the control treatment gave the lowest average of 129.2 µg /mL. The results indicate that there was a significant bi-interaction between the study factors in Coumaric acid concentration in *Gardenia jasminoides* L. callus where the interaction (LED + Putrescine with a concentration 1 mg/L) gave the highest mean average of 176.9 µg /mL while the combination (Florescent + 0) gave the lowest average of 122.4 µg /mL.

The results of the statistical analysis in Table (3) indicate a significant difference in the concentration of Ferulic acid in the *Gardenia jasminoides* L. callus, where the LED lighting treatment gave the highest average of 116.2 µg /mL, while the Florescent treatment gave the lowest average of 109.4 µg /mL. The treatment with Putrescine significantly affected the concentration of Ferulic acid in *Gardenia jasminoides* L. callus, where it gave the treatment at a concentration of 0.5 mg/L, the highest average of 120.9 µg /mL, while the control treatment gave the lowest average of 97.8 µg /mL. The results indicate that there was a significant bi-interaction between the study factors in the Ferulic acid concentration in the *Gardenia jasminoides* L. callus, where the interaction (LED + Putrescine at a concentration of 0.5 mg/L) gave the highest average of 126.0 µg /mL while the combination (Florescent + 0) gave the lowest average of 94.9 µg /mL.

Table (3) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Ferulic acid

Lighting Type	Putrescine (mg.L^{-1})				Mean
	0	0.5	1	1.5	
Florescent	94.9	115.7	117.5	109.4	109.4
LED	100.7	126.0	123.8	114.5	116.2
Mean	97.8	120.9	120.7	111.9	
L.S.D$_{(0.05)}$	Lighting 116.2	Putrescine 20.17	Lighting *Putrescine 28.52		

The results of the statistical analysis in Table (4) indicate the presence of significant differences in the concentration of Caffeoylquinic acid in *Gardenia jasminoides* L. callus, where the LED lighting treatment gave the highest average of 291.4 µg/mL while the Florescent treatment gave the lowest average of 276.3 µg/mL.

Table (4) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Caffeoylquinic acid

Lighting Type	Putrescine (mg.L^{-1})				Mean
	0	0.5	1	1.5	
Florescent	263.5	287.6	276.4	277.7	**276.3**
LED	272.1	307.7	296.0	290.1	**291.4**
Mean	267.8	297.6	286.2	283.9	
L.S.D$_{(0.05)}$	Lighting 24.06	Putrescine 34.03	Lighting *Putrescine 48.13		

The treatment with Putrescine significantly affected the concentration of Caffeoylquinic acid in *Gardenia jasminoides* L. callus, where the Putrescine treatment at a concentration of 0.5 mg/L was given at the highest average of 297.6 µg/mL, while the control treatment gave the lowest average of 263.5 µg/mL The results indicate that there was a significant bi-interaction between the study factors in the concentration of Caffeoylquinic acid in the *Gardenia jasminoides* L. callus, where the interaction (LED + Putrescine at a concentration of 0.5 mg/L) gave the highest average of 307.7 µg/mL while the combination (Florescent + 0) gave the lowest average of 263.5 µg/mL.

The results of the statistical analysis in Table (5) indicate a significant difference in the concentration of Sinapic acid in *Gardenia jasminoides* L. callus, where the LED lighting treatment gave the highest average of 262.8 µg/mL, while the Florescent treatment gave the lowest average of 257.7 µg/mL. The Putrescine treatment significantly affected in the concentration of Sinapic acid in *Gardenia jasminoides* L. callus, where the treatment at a concentration of 0.5 mg/L was given at the highest average of 274.5 µg/mL, while the control treatment gave the lowest average of 236.8 µg /mL. The results indicate that there was a significant bi-interaction between the study factors in the concentration of Sinapic acid in the *Gardenia jasminoides* L. callus, where the interaction (LED + Putrescine at a concentration of 0.5 mg/L) gave the highest average of 285.7 µg /mL while the combination (Florescent + 0) gave the lowest average of 232.9 µg /mL.

Table (5) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Sinapic acid

Lighting Type	Putrescine ($mg.L^{-1}$)				Mean
	0	0.5	1	1.5	
Florescent	240.7	263.3	264.5	262.2	257.7
LED	232.9	285.7	266.3	266.5	262.8
Mean	236.8	274.5	265.4	264.3	
$L.S.D_{(0.05)}$	Lighting 26.43	Putrescine 37.38	Lighting *Putrescine 52.86		

The results of the statistical analysis in Table (6) indicate the presence of significant differences in the concentration of Tannic acid in *Gardenia jasminoides* L. callus, where the LED lighting treatment gave the highest average of 87.9 µg /mL, while the Florescent treatment gave the lowest average of 80.8 µg /mL.

Table (6) The effect of the Lighting type and putrescine on the *Gardenia jasminoides* L. callus content of Tannic acid

Lighting Type	Putrescine ($mg.L^{-1}$)				Mean
	0	0.5	1	1.5	
Florescent	75.5	81.4	83.7	82.4	80.8
LED	80.8	83.7	94.6	92.7	87.9
Mean	78.2	82.6	89.1	87.6	
L.S.D(0.05)	Lighting 6.72	Putrescine 9.51	Lighting *Putrescine 13.45		

The treatment with Putrescine significantly affected the concentration of Tannic acid in *Gardenia jasminoides* L. callus, where the treatment was given at a concentration of 1 mg /L at the highest average of 89.1µg / mL, while the control treatment gave the lowest average of 78.2 µg /mL. The results indicate that there was a significant bi-interaction between the study factors in the concentration of Tannic acid in the *Gardenia jasminoides* L. callus, where the interaction (LED + Putrescine at a concentration of 1 mg/L) gave the highest average of 94.6 µg /mL, while the combination (Florescent + 0) gave the lowest average of 75.5 µg /mL.

The results showed that there was a significant overlap between the study factors in the concentration of Sinapic acid in *Gardenia jasminoides* L. Callus. The interference (LED + Putrescine at a concentration of 1) gave the highest mean of 94.6 $\mu g.ml^{-1}$ while (Florescent + 0) gave the lowest mean of 75.5 $\mu g.ml^{-1}$.

Of the results achieved, the increase in the concentration of phenolic compounds in *Gardenia jasminoides* L. Callus can be attributed to the effect of the type of lighting used on LED lighting. The amount of light intensity resulting from it is higher than the normal light, turning 20% of the electric energy into light. It was found the does not consume energy emitted in heat and does not cause damage to the part outside the in vivo compared to the other type of fluorescence, which converts about 4% while the rest is dispersed as a heat, so the amount of light received by the part is projected on the food medium by taking the needs of light ideally It absorbs the maximum limits of the actual needs and therefore this is reflected on the nature of its growth [22], [23] also explained the effect of light source on plants outside the organism to its involvement in the process of metabolism and form morphology, which is reflected in the formation of vegetative parts. Also pointed out that light is an environmental factor that has a significant influence on the physiological responses of plants. It increases the ability to absorb growth regulators, especially cytokines, by the vegetative parts of plants. It also reduces the side effects that produce high levels of Oxytin and cytokines added to the dietary medium.

In addition, also [24] pointed out that is light has a direct effect on nutrient accumulation, which is positively reflected in increased vegetative growth as well as its role in increasing cell productivity of secondary compounds [25]. The results of the present study were consistent with those found by [26], which obtained the best growth in the development of the plant parts of the *Alternanthera sessilis* plant in a laboratory that used 16-hour LED lighting in red and blue of the solar spectrum.

The significant increase in the multivariate amino-bioterase effect may be due to its role in activating enzymatic antioxidants and increasing non-enzymatic antioxidant rates. It also plays a direct role in increasing the levels of nucleic acids and mineral nutrients. This increases the concentration of phenolic substances [10],[27] have shown that bioterinsin plays an important role in cellular stimulation to increase the concentration of secondary compounds, including phenolic compounds. It also protects the plasma membrane by preventing the formation of free oxygen radicals, which cause severe damage to cellular membranes, nucleic acids and proteins inside Plant cells.

4) Conclusions

From the results obtained, we can conclude that the treatment with LED light and the adding of Putrescine led to a clear increase in the concentration of phenolic compounds measured in the *Gardenia jasminoides* L. callus according to the conditions of the experiment. We recommend conducting further studies on other plants containing active substances and increasing the concentration of phenolic compounds under different conditions.

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