**How to Cite:**
Al-jabar, K. M. A., & Saadon, A. S. (2022). Isolation and Identification of Alternaria arborscens from corn grains and possibility controlling of it by using Agaricus bisporus filterate and sodium citrate and their toxin. *International Journal of Health Sciences*, *6*(S5), 3182–3193.
https://doi.org/10.53730/ijhs.v6nS5.9326

# Isolation and Identification of *Alternaria arborscens* from corn grains and possibility controlling of it by using *Agaricus bisporus* filterate and sodium citrate and their toxin

**Khadija M. Abd Al-jabar**
College of Science/Al-Qadisiya University/Diwaniya/Iraq
Corresponding author email: Khadijamoussa196@gmail.com

**A.S. Saadon**
College of Science/Al-Qadisiya University/Diwaniya/Iraq

***Abstract*---**This study included isolating the accompanying and contaminated fungi of Corn Grains in the city of Al- Diwaniyah collected for the period September-December / 2021. A number of fungi were identified: of it *Aternaria arborscens.*, It was observed that the percentage of surface fungi associated with non-sterile Corn Grains samples was high. Fungal toxins were detected in the fungal leachates of one of these fungus *A. arborescens*was diagnosed with phenotype and genetically using PCR technology. So this species we have provided GenBank accession number(s) for it nucleotide sequence(s): ON386194 and ON386195 was recorded in Iraq in this study. The analysis was performed using HPLC technology showed results on the ability of *A. arborescens* to produce toxins. The highest concentration was the altratoxin which was 247.9 µg / ml. In the study *Agaricus bisporus* was used to conduct the biological resistance of pathogenic fungi to reduce the number and its toxicity of fungi *A. arborescens.* It is gave high resistance to *A. arborescens* when testing the contrast between them. The results of the study showed their ability to prevent the growth of pathogenic fungi in solid and liquid media. The study also identified the effect of chemotherapy sodium citrate on the radial growth in the center of the solid and dry weight in the liquid media. The results confirmed that the higher the concentration of the fungus leachates and sodium citrate in the culture media decreased the growth of pathogenic fungi, which means the inverse proportion between the articles and the growth of fungus pathogen. Results the test of the effect ofthe interference of the biomass and the sodium citrate in the growth of *A.arborescens,* the percentage of the colony diameter of the fungus was decreased The

inhibition rate was 80% at 30% and the dry weight was 92.59% at 30% the concentration of the interference treatments increased as the inhibition ratio increased Mushroom fungus.

***Keywords*---**Corn Grains, *Aternaria arborescens,* toxic fungal filtrate and *Agaricus bisporus*

## 1. Introduction

Corn (Zea mays), is an important food source as it is grown as acereal or forage plants and its edible grain. Is the third largest plant after wheat and rice in terms of production in the world, it is also the most consumed product in many countries (Cho *et al.* 2014). However, this plant is susceptible to infection by pathogenic fungi, which cause great losses to the crop as well as to the quality of food and commercial value. It is represented in the loss of dry matter or the inability to germinate, the breakdown of sugars, fats and proteins, and in particular it destroys the grain (Ristanovic,2001;Shurtelff,1984), they are also considered to be the first plant in the grasses to which a change was made in its genetic structure OGM. However, this plant remains vulnerable to infection by many non-living factors (drought and ocean pollution) and biotic (harmful insects and fungi). Abiotic factors affect the development of corn plant, which facilitates its infection with various fungal, bacterial diseases and viruses (Al-Amri ,2015).

Fungi are important pathogens, as grains are infected during harvest, transportation or storage by many fungal species that are naturally present in agricultural soils, the most important of which are *Alternaria, Ascochyta, Aspergillus, Fusarium, Penicillium, Pythium, Rhizoctonia, Rhizopus, Sclerotium, Uromyces* and other fungus causing cereal rots. Emergence and rotting of the roots and bases of the stems (Al-Jubouri and Jabr, 2012.), Among the plant pathogenic fungi known to produce toxins are isolates of the fungus *Alternaria* spp. It was found that they produce about forty compounds, most of which are toxic to organisms. These compounds were classified into groups based on their chemical composition, and their presence on stored grains leads to a decrease in grain germination, and changes Color, weight loss, putrefaction, in addition to the possibility of excreting toxins such as Alternariol, which are chemical compounds of low molecular weight (Ballio, 1991). Which cause serious diseases for both humans and animals (Christensen and Kaufman, 1969),*A. arborescens* isolates are effective pathogen in a cool weather of 10-25° C and fairly high humidity which consider a potential pathogen on Corn Grains plants. Agricultural and research institutions tended to find an alternative method for chemical pesticides, represented by biological resistance, as this method was good by not leaving toxic residues with an impact on the ocean and human health, and high efficiency in reducing the impact of plant pathogens (Montealegre *al et.*, 2003), characterized by It is also easy to use and not limited to a specific time (Lee et Lee, 2007), The search has also increased to find new alternatives such as Mushroom, which has achieved impressive results as a natural source of antibiotics, as the secretions cellular extra of the mycelium of mushrooms are effective and very resistant to bacteria and fungi (Hess *et al.,* 2018), including *Agaricusbisporus* ( The white button mushroom) is a staple in the economy and the beneficial effects of this type of mushrooms have been known for a long time, improving immune function (Solano-Aguilar *et al.*, 2018).

Due to the lack of local studies in this field, what has prompted us to strive with the unremitting efforts made and continuous attempts by researchers to do so by investigating the study of the contamination of the types of corn grain used in feeding with fungi, especially those producing toxin , and the possibility of controlling the toxin-producing by *Alternaria arborescens using some chemical and biological transactions*.

## 2. Methods

### Isolation and diagnosis

Samples of Corn Grains were collected from the markets and shops of Diwaniyah provincial center and districts for the period (September-December 2021). The contaminated fungi of grains used in this study were isolated where each type of sample was divided into two groups, the first group included samples that were superficially sterilized by sodium hypochlorite at a concentration of 1% and for 3 minutes after which they were washed with distilled water three times to remove the effect of sterilization Saduoon, (2005).The second group was washed with only distilled water, Parts of sterile and unsterilized samples were grown in petri dishes container on the PDA food medium by three repeaters per sample and the dishes were incubated for 7 days at 25 ℃ temperature, during which time the growth of fungi was followed up and the dishes were examined to find out the developing fungi, after which the appearance and microscopy of fungal isolations was diagnosed and confirmed this diagnosis molecularly. It is worth mentioning that the diagnosis of fungus*A. bisporus*was done with the help of references and scientists in this field, and was confirmed through the information available at the National Center for Biotechnology Information (NCBI).

### Detection of the toxic fungal filtrate

The analysis was carried out in the laboratories of the Department of Environment and Water of the Ministry of Science and Technology, as the toxins produced by some types of fungi were detected using the HPLC device model Skyam, and using a vector phase consisting of (Mobile phase = D.W: 5% Formic acid: methanol (20: 5: 75) and a C18-ODS (25 cm4.6 mm) separation column using Fluorescence detection and the mobile phase. (Flow rate = 1ml/min) and after conducting the analysis of the standard substance that was prepared by taking 0.1 g of the standard poison and dissolving it in a volume of 250 ml in a volumetric vial of 250 ml capacity, if the initial concentration was 40 µg/ml or 40 ppm, a concentration of 10 ppm was prepared by taking 0.4 ml from the initial concentration and complete the volume to 10 ml in a volumetric vial of 10 ml. 100 µl was taken from the last concentration and injected into a HPLC device under the same conditions (Tolgyesi et al., 2015), and using the following equation described by Akiyma and Chen (1999), The concentration of toxins was calculated in the fungus filtrate samples by the standard formula is C = m/V, where C is the concentration, m is the mass of the solute dissolved, and V is the total volume of the solution.

### Preparation of Fungal Culture Filterate

The filtrate was prepared by *A. arborescens* and *A.bispporus*, according to the method (Al-Shibli, 1998).Using liquid food medium PDB in conical glass flasks of a capacity of 250 ml and with an amount of 50 ml of culture medium for each beaker and were sterilized at a temperature ° 121 °C and under pressure of 15 pounds / inch 2 for a period of 15 minutes

and after cooling the medium, the antibiotic chloromphenicol was added and then the beakers were inoculated by placing two tablets of diameter (5) mm of known 7-day-old mushrooms in each beaker, and then the flasks were incubated in the incubator at 25°C for three weeks with shaking every 2-3 days. After the incubation period, the fungal cultures of *A. bisporus* were filtered using Whatman filter paper. No.1 using a sterile Buchner funnel and under sterile conditions, then sterilize the filtrate using Millipore Filters with a diameter of 0.22 microns and keep the filtrate until use at a temperature of 4°C

**The effect of the biological resistance (A bisporus ) on the growth of toxic fungus on the PDA medium**

Double Culture technique was used in 9cm petri dishes containing the solid food medium prepared in the PDA (Potato Dextrose Agar), with the use of the antibiotic Chloramphenicol by (250 mg/L) and to know the antagonistic ability of fungus*A. bisporus* and toxic fungus *A. arborescens* the dish was divided into two equal parts and using the mortal drill we work a hole in the first half and it was vaccinated with a( 5 mm )disc of fungus *A.bisporus* aged 7 days, He made a hole in the center of the other half of the dish and was vaccinated with a (5 mm) disc of 7-day-old toxic fungus and three repeaters, and the comparative treatment dishes contained each mushroom individually, and then incubated the dishes in the incubator at a temperature of 25°C and for 7 days and after the end of the incubation period calculated the degree of contrast for each fungus and according to the five standardization ladder mentioned by Bell et al., (1982).

**Effect of *A bisporus* filtrate on radial growth of the toxic fungus *Alternaria arborescens* on PDA medium**

Determining the effectiveness of*A. bisporus* tested in the radial growth of the *A. arborescens* followed the Dixit et al method, (1976) Poisoned Food Technique if three concentrations of*A .bisporus* filtrare, 10.20.30% of the sterile food medium Potatos Dextrose broth , move the medium a spiritual movement and then leave the dishes to harden and then by the mortal drill a piece of toxic fungus growth was made, Then a piece measuring (5) mm from the end of the radial growth of the *A. arborescens* and at the age of 7 days was transferred to the center of the dish and three repeaters per concentration and placed in the incubator for (7 days) at a temperature of 25 100 m, but the comparison treatment included dishes without adding the *A.bisporus* filtrate . After the completion of the incubation phase, the growth rate of fungus in the transactions of different compositions was measured 30,20,10% taking the growth rate of laboratory fungus in dishes using the ruler and after the fungal yarn reached the edge of the dish in the comparison treatment, radial growth was calculated by taking the growth rate of perpendicular diameters of developing colonies and then calculating the percentage of inhibition (percent inhibition of radial growth) .
By applying the equation, (Abbott,1925) mentioned by Shaaban and The Navigator, 1993) and used by Al-Musawi (2013).

$$\text{Inhibition percentage} = \frac{\text{Average weight of fungus in comparison} - \text{average weight of comparative fungus}}{\text{Average weight of comparative fungus}} \times 100$$

**Effect of sodium citrate concentrations on radial growth of toxic fungus*A. Arborescens***

to determine the effectiveness of sodium citrate tested in the radial growth of fungus*A. Arborescens* followed dixit et al. (1976), poisoned food technique, if three concentrations of sodium citrate salt were prepared: (30.20.10 mg/ml), the concentration is prepared 10% by taking (1g) of salt and dissolving it in 100 ml of sterile implant medium (PDA) Potatos Dextrose Agar, To prepare the concentration 20% by taking (2g) of salt is taken and dissolved in 100ml of sterile implant medium and so on for a concentration of 30% and followed the the sam previous steps**.**

**The effect of sodium citrate interference and A. bisporus filtrate in the radial growth of toxic fungus*A. arborescens***

to determine the effect and effectiveness of tested chemicals and filtrate of bio-resistance fungus *Aarborescens*, the intervention process was performed as follows:
5% of mushroom filtrate *A bisporus* + 5 mg/ml of sodium citrate.
10% of mushroom filtrate*A. bisporus* + 10mg/ml of sodium citrate.
15% of mushroom filtrate*A. bisporus* + 15mg/ml of sodium citrate.
As for the comparison treatment, the solid food medium was used without any addition to the transactions, and the same steps were followed .

**Effect of bio-resistant fungus *A. bisporus* in the dry weight of the toxic fungus in the liquid medium PDB**

to test the effectiveness of fungus filtrate *A. bisporus* in the dry weight of the toxic fungus A. arborescens,250ml conical flasks were used to prepare three concentrations for fungus filtrate *A. bisporus* which is (30.20.10%) of the liquid food medium PDB( Potatos Dextrose Broth) prepared and the center implanted by 50 ml per flask, By means of the mortal drill transfer of two discs measuring (5 mm )from the end of the radial growth of the fungus *A. arborescens* age 7 days to each flask, and with three repeats per concentration and placed in the incubator for 7 days at a temperature of (25C )˚, but control flasks without any addition, the flasks were shaken every two days and after the completion of the incubation period the dry weight fungus were measured in different concentrations using the sensitive balance, If the liquid medium is filtarated with filtration paper, the papers conveyed the weight in advance to the electric oven with a temperature of (60 C ˚) for 24 hours until it fully dry, then weighed the filtration papers and put the weight of the filtration paper before use:
(weight of the filtration paper after drying - weight of the filtration paper before use - the weight of the insolent),
We obtain the exact dry weight in the liquid culture medium and according to the percentage of inhibition (Pintoet .al .,2001)as in the following equation:

$$\text{Inhibition percentage} = \frac{\text{Average weight of fungus in comparison} - \text{average weight of comparative fungus}}{\text{Average weight of comparative fungus}} \times 100$$

**Effect of sodium citrate on the dry weight of the toxic fungus *A.arborescens* in the liquid mediumPDB**

to test the effectiveness of chemicals in the dry weight of fungus *A. arborescens* attended three concentrations of sodium citrate (30.20.10g/ml) by taking (3.0, 2.0, 1.0 ) and dissolving them in 100 ml of pre-prepared PDB food medium and following the same steps

**The effect of interactionbetween sodium citrate and mushroom filtrate on the dry weight of *A. arborescens*in amedium PDB**

To test the interaction effectiveness between the filtarate*A.bisporus*and sodium citrate in the dry weight of *A. arborescens* tested in the liquid medium used flasks 250 mL for this purpose , the liquid medium (PDB)was placed Three concentrations of fungus filtrate and sodium citrate were prepared from the sterile liquid medium . The process was carried out as in the previous steps.

**Statistical analysis**

The results of the entire study were analyzed using the statistical software GraphPad Prism Institute, Inc. According to the data of the results of the study, the statistical test Chi-square test and the method of least significant difference (LSD) for one-way variance and two-way variance were applied to determine the least significant differences between study groups, as well as the adoption of a confidence interval equal to 95% and a value at the probability level less than 0.05 ($P < 0.05$) (Motulsky, 2003).

**Results and Discussion**

Primarily, to identify the pathogen, phenotypical identity of the isolates was adopted on PDA medium and under standardized conditions. So The different species belonging to the genus *Alternaria* sp. were the most abundant in maize samples, and of these isolated fungi is *A. arborescens*, and it had a relative frequency of 5.4% among the fungi isolated in the superficially unsterilized samples, while the relative frequency was 8.8% in the superficially sterilized, which is The highest compared to the rest of the fungi, and the conidia of the contaminated fungus appear (Fig. 1) as it was found that it matches somewhat what was mentioned in a number of relevant literature (Simmons & Roberts, 1993; Rao et al., 2017; Ramezani et al., 2019). The colony was usually dark olive gray with white margin. Conidia were ovoid in shape with dark brown colour (11 to 33 × 6.0 to 14.0 µm). Conidiophores had narrow and spire configuration, 50 to 200 × 2.5 µm. This species was diagnosed morphologically and microscopically and these two diagnoses were confirmed through the molecular diagnosis procedure using polymerase chain reaction technology (PCR) where the results of this analysis proved that this isolation belongs to type *A. arborescens*. By comparing the sequence of nitrogen bases from isolated *A. arborescens* fungus in

this study, it was found that there was a 100% similarity to many *A. arborescens* fungus isolates previously registered at the National Center for Biotechnology Information (NCBI). So this species we have provided GenBank accession number(s) for it nucleotide sequence(s): ON386194 and ON386195was recorded in Iraq in this study. This is consistent with the findings of Al-Zayadi (2011), which showed the success of this fungus's resistance in different environments and its high virulence represented by causing large percentages of infection in corn grains, and that it has a high competitive ability against a number of other fungi that pollute seeds. It was brought by Leticia *etal.,* (2007) who showed that the highest percentage frequency of the fungi contaminating seeds and excreting the toxin \was *Alternaria* spp.

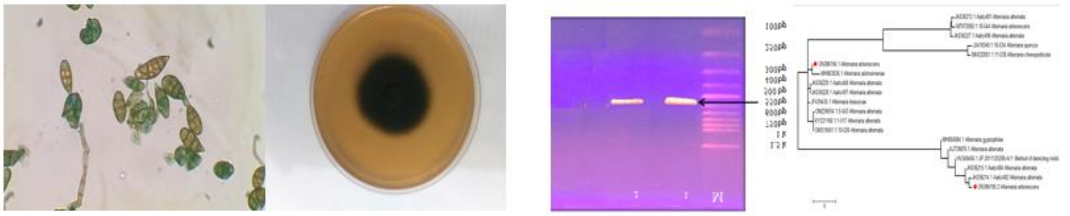

Figure (1) *A. arborescens* isolated from maize grain isolated on PDA medium, under a light microscope, the products of electrophoresis on agarose gel (1%) for the 18 S rRNA region (bp 550) and the genetic tree using Mega 6 program, of *A. arborescens* isolated in Iraq respectively

The results showed the use of high-performance liquid chromatography (HPLC) to detect fungus toxic*A. Arborescens* filtrate .The value of the detention time is (3.37) minutes as it represents the standard area of the toxins, and these values indicate the presence of toxic fungus filtrate toxins in the examined isolates based on matching the detention time of the standard substance with the time of appearance of these values for the same conditions in which the analysis of the standard substance was conducted. The results showed that the toxic fungi had the ability to secrete mycotoxins in all samples, as the highest concentration of the toxin produced from the filter of the selected fungus isolate *A. arborescens* for the sample of Argentine yellow corn was 247.9 g/ml, and the lowest concentration recorded in the sample of Syrian sorghum was the mycotoxins 152. 6 g /ml. The results of the experiments related to studying the antagonistic ability of *A.bisporus* against the toxic fungus isolated from the corn grain samples shown in Figure (2) showed the ability of *A.bisporus* to completely inhibit the growth of *A. arborescens,* as it is noted on P.D.A culture medium. The antagonism of *A bisporus* from the second degree according to the five-step scale of standardization mentioned by (Bell *et al.*, 1982)

Form (2) antagonism between *A. arborescen* and *A. bisporus*. After (7) days of incubation

Showed a statistical analysis of the results of the inhibitory effect of *A.bisporus* to *A. arborescens* tested, all concentrations used, that there are significant differences ( $P < 0.05$ ) according to the concentration. As the rates of fungal colonies diameters were inversely proportional to the concentration of the transaction table (1)
The study also confirmed the results of the current table (2) the results of the effect of various transactions in dry weight of the tested fungi are highly significant in relation to the comparison operators.

Table (1) Effect of A. bisporus filtrate and sodium citrate on the radial growth of *A. arbrorescens* in PDA medium

| Concentration mg/mland percentages of dilutions | Treatment *A.bisporus* | | Treatment sodium citrate | | Interference treatment between sodium citrate&*A.bisporus* | | Concentration inhibition rate ± standard deviation |
|---|---|---|---|---|---|---|---|
| | Diameter (cm) | Inhibition % | Diameter (cm) | Inhibition % | Diameter (cm) | Inhibition % | |
| % 10 | 3.25 ±0.13 | 50 | 3.2 ±0.05 | 44.82 | 2.63 ±0.27 | 54.65 | 2.02 ±0.33 |
| % 20 | 2.8 ±0.22 | 56.92 | 2.26 ±0.41 | 61.03 | 2.11 ±0.33 | 63.62 | 2.39 ±0.42 |
| % 30 | 1.27 ±0.12 | 80.46 | 1.2 ±0.08 | 79.31 | 1.16 ±0.1 | 80 | 1.21 ±0.10 |
| Control | 6.5 ±0 | - | 5.8 ±0 | - | 5.8 ±0 | - | 6.03 ±0.35 |
| Inhibition rate coefficients ± deviation | 3.45 ±1.99 | | 3.11 ±1.78 | | 2.92 ±1.82 | | |
| L.S.D . value | for concentration0.166 | | for transactions0.192 | | to interfere 0.333 | | |

The anti-fungal activity of the chemical sodium citrate is attributed to the fact that it is a basic salt behavior; it affects the pH value of the medium towards the basal, which negatively affects the growth of the fungus, thus inhibiting its growth . These results are consistent with the findings ofAhuroni*et al.*, (1997) showed that sodium bicarbonate showed a high inhibitory ability of the fungi *Alternaria alternata*, *Fusarium*.sp and *Rhizopus stolonifer*, and agreed with what Sivakumar*et al.*, (2002) reported that sodium bicarbonate and sodium citrate inhibited the radial growth and fungus spores germination of *Colletotrichumgloeosporioides*.

The results of the study are in agreement with what was reported by Chowdhary*et al.* (2015) that the species belonging to the mushrooms are characterized by

containing many antimicrobials, antioxidants, biological active substances, and a high susceptibility to colonization and rapid growth of the fungus on agricultural media, and this was mentioned by Vaman, (2012). It also agrees with what was indicated by Timoz (2015) on the ability of the fungus *A .bisporus* to inhibit the growth of some toxic fungi, including *A. arborescens.*

Table (2) Effect of *A. bisporus filtrate and* sodium citrate on the dry weight of *A.arborescens* in liquid media PDB

| Concentration mg/ml and percentages of dilutions | Treatment *A.bisporus* | | Treatment sodium citrate | | Interference treatment between sodium citrate&*A.bisporus* | | Concentration inhibition rate ± standard deviation |
|---|---|---|---|---|---|---|---|
| | Weight (gm) | Inhibition % | Weight (gm) | Inhibition % | Weight (gm) | Inhibition % | |
| % 10 | 0.12 ± 0.03 | 52 | 0.15 ± 0.03 | 31.81 | 0.1±0.02 | 62.96 | 0.123±0.02 |
| % 20 | 0.08 ± 0.02 | 68 | 0.09 ± 0.01 | 59.09 | 0.09 ± 0.01 | 66.66 | 0.086±0.005 |
| % 30 | 0.04 ± 0.001 | 84 | 0.04 ± 0.002 | 81.18 | 0.02 ± 0.003 | 92.59 | 0.033±0.01 |
| Control | 0.25±0.03 | - | 0.22 ± 0.04 | - | 0.37±0.04 | - | 0.24±0.08 |
| Inhibition rate coefficients ± deviation | 0.12 ± 0.03 | | 0.15 ± 0.03 | | 0.120±0.10 | | 0.123±0.02 |
| L.S.D . value | for concentration0.0012 | | for transactions 0.0017 | | to interfere0.0025 | | |

The results of the Al-Zubaidi study (2015) showed that both aqueous and alcoholic extracts of *A.bisporus* have great antagonistic activity against all types of Gram-negative and Gram-positive bacteria by diffusion well Agar, indicating that the extracts have a broad spectrum.We conclude from this that the biological treatment has caused a significant increase in the general performance of the chemical, but sodium citrate has caused a smaller increase in the performance of the biological resistance. These results are in agreement with what Hassan (1985) found in the efficiency of the chemical pesticide in suppressing the activity of the fungi *Fusarium* sp, *Rhizoctonia solani Ulocladium* sp. as well as with what was found (Jones, 1999) in the efficiency of the pesticide against the fungus *F. graminearum* transmitted by wheat seeds on controlling diseases Maize root rot caused by the fungus *F. graminearum. F.moniliforme.*( Pal et al., 2001) also found the efficacy of the bacteria Pseudomonas fluorescens and against the fungus *Rhizoctonia solani.* A number of researchers (Cook *et al.*, 2002) also found the possibility of mixing organisms with number of fungicides.These results explain that the fungus *A.bisporus* filtrate contains effective compounds, phenolic compounds that inhibit the enzymes responsible for basic metabolic reactions through their unspecialized interaction with proteins, which leads to protein transcription and thus the inability of microorganisms to continue (Al-Lishi *et al.,* 2009).

**Reference**

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. J. econ. Entomol, 18(2), 265-267.

Al- Shibli, MajedKazemAbbod .Biological resistance of pathogenic fungi and secondary fungi associated with Master thesis . Faculty of Education . Al – Qadisiyah University.

Al-Ameri, W., Teklu, T., Graves, R., Kazemi, H., &AlSumaiti, A. (2015, April). Low-salinity water-alternate-surfactant in Low-permeability Carbonate Reservoirs. In IOR 2015-18th European Symposium on Improved Oil Recovery (pp. cp-445). European Association of Geoscientists & Engineers.

Al-Saidi, G. H., &Saadon, A. A. S. (2019, September). The study of toxic effects of toxic isolate Alternariaalternata in vivo of white mice and the ability of Biological and Chemical treatments in the reduction of toxicity. In Journal of Physics: Conference Series (Vol. 1294, No. 6, p. 062015). IOP Publishing

Al –Jubouri , Iyad Waleed Abdullah .(2011).Improving the productivity and treasury capacity of by using mineral and organic nutrients ,Iraq Journal of Agricultural Sciences .42 (6);65-72

Ballio, A. (1991). Phytotoxins and their involvement in plant diseases. Experientia, 47, 751-826.

Bar-Yossef, Z., Jayram, T. S., Kumar, R., Sivakumar, D., &Trevisan, L. (2002, September). Counting distinct elements in a data stream. In International Workshop on Randomization and Approximation Techniques in Computer Science (pp. 1-10). Springer, Berlin, Heidelberg.

Behboudi-Gandevani, S., Amiri, M., BidhendiYarandi, R., &RamezaniTehrani, F. (2019). The impact of diagnostic criteria for gestational diabetes on its prevalence: a systematic review and meta-analysis. Diabetology& metabolic syndrome, 11(1), 1-18.

Chaichan, M. T., & Al-Zubaidi, D. S. M. (2015). Control of hydraulic transients in the water piping system in Badra–pumping station No. 5. Al-Nahrain Journal for Engineering Sciences, 18(2), 229-239.

Chen, Z. S., Kawabe, T., Ono, M., Aoki, S., Sumizawa, T., Furukawa, T., ... & Akiyama, S. I. (1999). Effect of multidrug resistance-reversing agents on transporting activity of human canalicularmultispecific organic anion transporter. Molecular Pharmacology, 56(6), 1219-1228.

Cho, K., Van Merriënboer, B., Bahdanau, D., &Bengio, Y. (2014). On the properties of neural machine translation: Encoder-decoder approaches. arXiv preprint arXiv:1409.1259.

Chowdhury, S. P., Hartmann, A., Gao, X., &Borriss, R. (2015). Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42–a review. Frontiers in microbiology, 6, 780.

Christensen, C. M., & Kaufman, H. H. (1969). Mycotoxins and grain quality. Grain Storage-The Role of Fungi in Quality Loss." Univ. Minnesota Press, Minneapolis, 76-93.

Cook, E. R., Palmer, J. G., &D'Arrigo, R. D. (2002). Evidence for a 'Medieval Warm Period'in a 1,100 year tree-ring reconstruction of past austral summer temperatures in New Zealand. Geophysical Research Letters, 29(14), 12-1.

Dixit, A. (1978). The balance of trade in a model of temporary equilibrium with rationing. The Review of Economic Studies, 45(3), 393-404.

Gomes, J., Al Zayadi, A., & Guzman, A. (2011). Occupational and environmental risk factors of adult primary brain cancers: a systematic review.

Hassan, S. A., Bigler, F., Blaisinger, P., Bogenschütz, H., Brun, J., Chiverton, P., ... & Van Zon, A. Q. (1985). Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the

IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. Eppo Bulletin, 15(2), 214-255.

Hess Jr, R. L., Ganesan, S., & Klein, N. M. (2003). Service failure and recovery: the impact of relationship factors on customer satisfaction. Journal of the academy of marketing science, 31(2), 127-145.

Jones, K. C., & De Voogt, P. (1999). Persistent organic pollutants (POPs): state of the science. Environmental pollution, 100(1-3), 209-221.

Lee, J. A., Carvalho, C. M., &Lupski, J. R. (2007). A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. cell, 131(7), 1235-1247.

Leticia E.B., Hector , Silvia and Ana (2007). Alternariaalternata Prevalence in cereal grains and soybean seeds from Entre Rios, Argentina. Rev IberoamMicol ; 24:47-51.

Montealegre, J. R., Reyes, R., Pérez, L. M., Herrera, R., Silva, P., &Besoain, X. (2003). Selection of bioantagonistic bacteria to be used in biological control of Rhizoctoniasolani in tomato. Electronic Journal of Biotechnology, 6(2), 115-127.

Motulsky, H. J. (2003). Prism 4 statistics guide—statistical analyses for laboratory and clinical researchers. GraphPad Software Inc., San Diego, CA, 122-126.

Pinto, V. F. (2008). Detection and determination of Alternariamycotoxins in fruits and vegetables. In Mycotoxins in fruits and vegetables (pp. 271-278). Academic Press.

Sadoon, Abedelamer. (2005).Using jet root powder and sodium hypochlorite as alternatives to using chemical pesticides to combact fungi associated with wheat seeds before planting .Al-Qadisyah Journal of Pure Sciences .Environmental Research Issue .volume (10).

Sengupta, A., Pal, T. K., &Chakraborty, D. (2001). Interpretation of inequality constraints involving interval coefficients and a solution to interval linear programming. Fuzzy Sets and systems, 119(1), 129-138.

Sieber, C. M., Probst, A. J., Sharrar, A., Thomas, B. C., Hess, M., Tringe, S. G., &Banfield, J. F. (2018). Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. Nature microbiology, 3(7), 836-843.

Rinartha, K., Suryasa, W., & Kartika, L. G. S. (2018). Comparative Analysis of String Similarity on Dynamic Query Suggestions. In *2018 Electrical Power, Electronics, Communications, Controls and Informatics Seminar (EECCIS)* (pp. 399-404). IEEE.

Solano-Aguilar, G. I., Lakshman, S., Jang, S., Gupta, R., Molokin, A., Schroeder, S. G., ... & Urban, J. F. (2021). The Effects of Consuming White Button Mushroom Agaricusbisporus on the Brain and Liver Metabolome Using a Targeted Metabolomic Analysis. Metabolites, 11(11), 779.

Timoz.SolafHamed .(2012) molecular diagnosis of fungus *Pleurotusostr*eatus and the effectiveness of som industrial waste in its productions and ability to store .PhD thesis. College of Educati, University of Al-Qadisiyah .

Tölgyesi, Á., Stroka, J., Tamosiunas, V., &Zwickel, T. (2015). Simultaneous analysis of Alternaria toxins and citrinin in tomato: An optimised method using liquid chromatography-tandem mass spectrometry. Food Additives & Contaminants: Part A, 32(9), 1512-1522.

Tripathy, D. N., Hanson, L. E., & Mansfield, M. E. (1976). Evaluation of the immune response of cattle to leptospiralbacterins. American Journal of Veterinary Research, 37(1), 51-55.

Vaman, D. (2012, October). TRN history, trends and the unused potential. In 2012 IEEE/AIAA 31st Digital Avionics Systems Conference (DASC) (pp. 1A3-1). IEEE.