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Conference Paper *in* IOP Conference Series Earth and Environmental Science · December 2023

DOI: 10.1088/1755-1315/1262/3/032005

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The Efficiency of Fungicides on Fungal Growth Inhibition in Cultured Tissues of Banana *(Musa acominata*), and Their Effect on Tissues Traits

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Abstract. The goal of this study was to identify fungicides with a broad-spectrum effectiveness and their capacity to inhibit fungal contaminants in banana tissue cultures. Numerous fungi were isolated from tissues of banana cultures of the Grand 9 variety. *Aspergillus flavus, Aspergillus niger, Cladosporium oxysporum, Penicillium digitatum, P. expansum, and Penicillium* sp. were among the contaminated fungi isolated. These fungi were inhibited successfully using the fungicides Beltanol, Agrisave, and Zoxis. Hoever, Beltanol was outperformed compared with Agrisave and Zoxis fungicides, with an inhibition rate of 100% for concentrations of 0.25, 0.5, and 1 ml/l. Thus, the addition of Beltanol fungicide to the banana culture medium had a positive influence on the fresh and dry weight of the tissues, with the average fresh weight increasing to 2.93 g compared with 2.4 g in the control treatment. Also, the dry weight was increased from to 0.21 g while it was 0.17 g in the control treatment. Additionally, the results demonstrated the enzymatic effectiveness (cellulase enzyme) of isolated fungi without treatment, that showed high effectiveness in *P. digitatum* fungus with an activity rate reached 2.33 mm. In contrast, *C. oxysporum* and *P. expansum* fungi displayed moderate efficiency with activity rates that reached 1.5 and 1.41 mm respectively. On the other hand, *A. flavus* and *A. niger* fungi exhibited low efficacy with activity rates of 1.25 and 1.12 mm respectively. Furthermore, the results revealed that *P. expansum* fungus had a high enzymatic effectiveness index with a rate of 1.85 mm, while *C. oxysporum*, *P. digitatum*, and *A. flavus* fungi had moderate enzymatic effectiveness with rates of 1.46, 1.34, and 1.26, respectively. *A. niger* fungus had the lowest enzymatic effectiveness with a rate of 1.23. The enzymatic secretion of the isolated fungi was inhibited efficiently via addition of the fungicide Beltanol to these fungi.

Keywords. Banana, enzymes, fungicides, microbial contamination.

1. Introduction

The banana plant is a monocot plant that is split into the genera *Ensete* and *Musa* and is a member of the Musaceae family [1]. Banana has a high nutritional value and is highly desired by consumers due to its sweetness and distinct flavour, in addition to its economic importance to many countries [2]. The fruit is high in fibre, carbohydrates, vitamins, and minerals, particularly potassium, which is required

for basic cell functions [3]. Plant tissue culture, also known as micropropagation, has been used to create a variety of banana cultivars. This method entails obtaining pest-free sterilized plant parts, providing suitable temperature, light, and humidity conditions, and preparing an ideal growth environment that can be controlled [4,5]. The growth media used in plant tissue culture are also a good source of nutrients for microorganism growth. These microorganisms compete for nutrients with tissue farmers, and some produce toxins that cause crop death, tissue necrosis, and reduced reproduction and rooting [6,7]. Although biological contamination can occur from a variety of sources in tissue culture labs, fungal and bacterial contamination are the most serious problems encountered in micropropagation [8]. The risk of biological contamination stems from the negative competition between these microorganisms and plant growth, as the culture medium is a good source of food for these microorganisms, which can result in plant death or limited growth and rooting [9,10]. Because of the damage it causes and the speed with which it spreads, fungal contamination is the most dangerous because it can arise from excised parts, air, or during the propagation process [11]. Many fungicides have been used to control plant pathogenic agents, including dithiocarbamates and strobilurins [12]. Additionally, Azoxystrobin, Clothianidin, Beltanol. L Spinosyn, and Thiodicarb have also been used to control fungal diseases [13, 14]. Furthermore, fungicides such as Carbendazim and Score have been used to prevent contamination in tissue culture agriculture laboratories in Iraq, with good results and no side effects on plant tissue growth and development [15]. However, contamination problems, particularly fungal contamination, have recently increased due to a lack of appropriate pesticides to eliminate them. This is due to a number of factors, the most important of which are fungal resistance to chemical pesticides and the spread of fungal pesticides with numerous trade names that are incapable of controlling all of the fungi that cause contamination [16-18]. The use of pesticides to prevent contamination in tissue farms should be based on a prior study to choose the best pesticides in terms of their impact without affecting the plants. In general, this practice will lead to minimize usage of the fungicides [19, 20]. The current study has been performed to determine the most common fungal contaminants of Banana tissue cultures and the efficiency of some fungicides in their control.

2. Materials and Methods

2.1. Isolation and Diagnosis of Fungal Contaminants in Banana Tissue Cultures

Samples of tissue culture banana plants (Grand 9 variety) grown in culture media inside jars and contaminated with fungi were collected. The contaminated colonies were isolated on Potato Dextrose Agar (PDA) in sterilized petri dishes with three replicates for each fungus. The plates were incubated at 25°C in darkness, and after five days, a hypha tip of each growing colony was taken using a sterile needle. The morphological and microscopic characteristics were identified under a compound microscope (Biolab line-China) at a magnification of X40, according to the taxonomic keys mentioned in [21,22]. The isolates were stored on PDA culture media at 4°C until further experiments were conducted.

2.2. The Efficiency of some Fungicides in Inhibition of Fungal Growth

Prepared Potato Dextrose Agar (PDA) medium and sterilized in an autoclave steam sterilizer. After sterilization, the medium was left to cool down to pre-solidification temperature, which is approximately 45-50 °C. Then, the medium was distributed into sterilized glass flasks with a volume of 250 mL and a rate of 200 mL per flask. Concentrations of 0.25, 0.5, and 1 mL/L of Agrisave fungicide and concentrations of 0.25, 0.5, and 1 mL/L of Zoxis fungicide were added separately to the flasks containing the PDA medium. Concentrations of 0.25, 0.5, and 1 mL/L of Beltanol fungicide were added to the PDA medium before sterilization in the autoclave. The media containing the fungicides and the control medium without fungicides were poured into sterilized Petri dishes with a diameter of 9 cm for fungal isolation of *Aspergillus flavus, Aspergillus niger, Cladosporium oxysporum, Penicillium digitatum, Penicillium expansum,* and *Penicillium* sp. The plates were then incubated at 25 ±2ºC for seven days. Fungal growth was evaluated by measuring the diameter of the colony using two perpendicular diameters that pass through the center of the colony. The percentage of inhibition was calculated using the Abott formula as described previously [23].

$$\text{Fungal Growth inhibition \%} = \frac{\text{Fungus growth average in control} - \text{Fungus growth average in treatment}}{\text{Fungus growth average in control}} \text{x}100$$

2.3. The Effect of Beltanol Fungicide on the Number of Tissue Banana Buds of Grand9 Variety

The toxic media method [24] was applied by preparing the tissue culture medium for banana growth and 0.25 ml/L of Beltanol pesticide was added. Three replicates were prepared with the fungicide treatment and three replicates were left without the pesticide for comparison purposes. All jars were sterilized using an autoclave, left to cool and solidify, and then the banana tissue culture was planted inside a sterile cultivation room. All buds were removed from the banana tissue culture before planting. All jars were incubated at 27°C for 30 days, and the number of developing buds in each jar was counted and recorded for both the treatment and the comparison groups.

2.4. The Effect of Beltanol Pesticide on the Fresh and Dry Weight of Tissue-Cultured of Banana Grand 9 Variety

The tissue culture medium for banana was treated with Beltanol fungicide at a concentration of 0.25 ml/L. Three replicates of the treated medium were prepared along with three replicates of untreated medium as a control. All replicates were sterilized using an autoclave and left to cool and solidify [25]. The tissue culture was then planted on the medium in a sterile growth room after removing all buds. The replicates were incubated at 27 °C for 30 days, and the plant tissue was weighed using a sensitive balance to measure the fresh weight. Then, the tissue was dried in an oven at 65-70 °C for 72 hours and weighed again to measure the dry weight.

2.5. Enzymatic Activity of Fungi Isolated After Treatment with Beltanol Fungicide

Special culture media were used in this experiment to detect the extracellular enzymatic activity of contaminated fungi. Disks with a diameter of 0.5 cm were taken from all isolated fungi grown on PDA culture medium after 7 days. The plates containing the enzymatic test culture medium were inoculated with 3 replicates for each treatment. The plates were incubated at a temperature of 25 ± 2ºC, and the enzymatic activity was detected before fungal growth reached the edge of the plate. Cellulase enzymes were detected using the method described by [26], lipase enzymes were detected using the method described by [27], and protease enzymes were detected using the method described by [28].

2.6. Statistical Analysis

A Completely Randomized Design (CRD) was used in the laboratory experiments, and all means were compared using the Least Significant Difference (L.S.D) method at a significance level of 0.05. Statistical software programs, such as Genestat and Microsoft Excel, were used for data analysis and graphical representation.

3. Results and Discussion

3.1. Isolation and Identification of Fungi Contaminating Tissue Bananas

The Potato Dextrose Agar (PDA) medium was used to isolate a group of fungi contaminating tissue bananas from contaminated banana plantations in the tissue agriculture laboratory in Al-Haritha. The isolated fungi are the most common contaminants in tissue agriculture laboratories, which was confirmed by [29], who found that *Aspergillus niger*, *Penicillium* sp., and *Alternaria alternata* are among the most common fungi contaminating tissue agriculture. In another study, [30] isolated various fungal genera from contaminated tissue from date palm plantations, including *Aspergillus niger*, *Chaetomium atrobrunneum*, *Penicillium* sp., and *Fusarium* spp.

Table 1. Isolated Fungi from Tissue Culture Banana (Grand 9) Explants.

Isolation site	Isolated Fungi
Tissue Culture Agriculture Lab/Hartha	*Aspergillus flavus* *Aspergillus niger* *Cladosporium oxysporum* *Penicillium digitatum* *Penicillium expansum* *Penicillium sp.*

3.2. The Efficiency of some Fungicides in Inhibition of Fungal Growth

Table 2 shows that adding different concentrations of fungicides to the PDA culture medium inhibited the growth of contaminated fungi. Beltanol fungicide was the most effective at 100% inhibition of all tested fungi at all concentrations. The addition of Zoxis fungicide resulted in an average inhibition rate of 27% at a concentration of 0.25 ml/L, with significant differences when compared to other concentrations. The inhibition rate was similar and without significant differences at concentrations of 0.5 and 1 ml/L, reaching 24.66% and 24.16%, respectively. The results also revealed significant differences in the case of Agrisave fungicide, with a concentration of 0.25 ml/L yielding the highest inhibition rate of 26.166%, followed by a concentration of 0.5 ml/L providing an inhibition rate of 24.833%, and a concentration of 1 ml/L providing the lowest inhibition rate of 13.33%. These fungi were resistant to some fungicides but sensitive to others. Beltanol inhibits these fungi effectively because it affects the biological processes of fungal cells and their components in a variety of ways. This was confirmed by [31], who stated that fungicides have negative effects on the cell membranes of microorganisms, causing structural changes and destroying the fats within them, which affects the function of these membrane systems. The Agrisave and Zoxis fungicides showed varying degrees of resistance, which is consistent with many studies showing that many fungi have become resistant to fungicides, and the effectiveness of these fungicides is dependent on their composition, concentration, and fungus targeting method. Certain fungi have cell membrane structures that allow them to resist many fungicides [32]. These fungicides may vary in inhibition rates, as confirmed by [33,34], as the sensitivity of *P. digitatum* fungus may differ.

Table 2. The efficiency of some fungicides in inhibition of fungal growth (%).

Fungi	% inhibiting fungal growth Fungicides											
	Agrisave				Zoxis				Beltanol			
	0.25	0.5	1	fungus average	0.25	0.5	1	fungus average	0.25	0.5	1	fungus average
Aspergillus flavus	16	8	8	10.66	33	16	16	21.66	100	100	100	100
A. niger	8	8	16	10.66	16	16	33	21.66	100	100	100	100
Cladosporium oxysporum	33	33	16	27.33	33	16	16	21.66	100	100	100	100
Penicillium digitatum	20	20	20	20	20	40	20	26.66	100	100	100	100
P . expansum	40	40	10	30	20	40	40	33.33	100	100	100	100
Penicillium sp.	40	40	10	30	40	20	20	26.66	100	100	100	100
Concentrations average	26.1	24.8	13.3		27.0	24.6	24.1		100	100	100	
$L.S.D_{0.05}$	for fungi = 2.114 for concentration= 1.495 Interaction= 3.661				for fungi = 2.014 for concentration =1.455 Interaction = 3.563							

3.3. The Effect of the Beltanol on the Number of Tissue-Cultured Banana Buds of the Grand9 Variety

According to Table (3), adding the Beltanol to the banana tissue-culture medium resulted in differences in the number of buds, with the average number of buds in the Beltanol treatment being 2.6, significantly lower than the average number of buds in the control treatment, which was 6. This is due to the pesticide's effect on bud production by influencing protein biosynthesis within the plant, which is consistent with a study by [35] that found that adding pesticides to plants reduces the number of buds. Another study discovered that applying chemical pesticides to plants changed some of their characteristics, such as bud production[36].

Table 3. Effect of Beltanol pesticide on the number of tissue banana buds of Grand9 variety.

Treatment	Mean number of buds
Beltanol 0.25 mL/L	2.6
Control	6
$LSD_{0.05}$	1.118

3.4. The Effect of the Beltanol on the Fresh and Dry Weight of Tissue Bananas of Grand 9 Variety

Table (4) shows that treating tissue-cultured bananas with the fungicide Beltanol improved the average weight of both fresh and dry tissue without causing any significant differences in means. Bananas treated with Beltanol gained fresh weight, rising from 2.4 g in the control treatment to 2.93 g. The effect of Beltanol on the tissue's dry weight was also noticeable, with a significant increase from 0.17 g in the control treatment to 0.21 g in the Beltanol-treated group. This could be attributed to carbohydrate accumulation in plant cells as a result of the addition of Beltanol to the growth medium, resulting in enhanced biological processes. These results are in line with those of [37], who claimed that certain chemicals, such as pesticides, can enhance plant growth and characteristics, leading to improved fresh and dry weights. Additionally, [38] discovered that some fungicides can have a favorable impact on the development and characteristics of plants.

Table 4. Effect of Beltanol pesticide on the fresh and dry weight of tissue bananas of Grand 9 variety.

Treatment	Fresh Weight (g)	Dry Weight (g)
Beltanol	2.93	0.21
Control	2.4	0.17
$LSD_{0.05}$	0.526	0.044

3.5. Extracellular Enzymatic Activity of Isolated Fungi was Studied After Treatment with Beltanol Pesticide

According to Table (5)'s findings, the isolated fungi were capable of producing hydrolytic enzymes like cellulase, lipase, and protease. The cellulase enzyme assay results revealed that all of the fungi tested positive, but with varying abilities to produce this enzyme. A solid medium containing carboxylic compounds such as cellulose (CMC-Agar) was used, and the appearance of a yellow halo around the fungal colony indicated that this substance was being decomposed by the cellulase enzyme into simple sugars. The fungus *P. digitatum* produced this enzyme with high efficiency, with an activity rate of 2.33 mm, whereas the fungi *C. oxysporum* and *P. expansum* produced it with moderate efficiency, with activity rates of 1.5 and 1.41 mm, respectively. On the other hand, *A. flavus* and *A. niger* showed low efficiency with activity rates of 1.25 and 1.12 mm, respectively. These findings are consistent with those of [39], who discovered that when 61 fungal species were isolated from the root areas of different plants, the isolated fungi produced varying amounts of the cellulase enzymes. Furthermore, [40] demonstrated that 18 fungal species responded positively to the enzyme by forming a yellow halo around the fungal colonies, indicating the conversion of complex carbohydrates into simple sugars. The larger the halo, the greater the enzyme production. The protease enzyme assay results also revealed that *P. expansum* had a high efficiency indicator in the breakdown of protein on skim milk agar, with an activity rate of 1.85 mm. *C. oxysporum*, *P. digitatum*, and *A. flavus*, on the other hand, had moderate enzymatic efficiency rates of 1.46, 1.34, and 1.26, respectively. The fungus *A. niger* had the lowest enzymatic efficiency, with an activity rate of 1.23, which is consistent with the findings of [41], who discovered that these fungal species can produce protease enzymes. As [42]

demonstrated, these fungi can produce protease enzymes in agricultural environments. It has also been discovered that for all isolated fungi, the enzyme lipase forms a white precipitate under the fungal mycelium, with white crystals submerged in the growth medium. The visible precipitate formed around the fungal colony due to the release of calcium salts from lactic acid indicates the positive activity of the lipase enzyme [43]. The isolated fungi's ability to secrete enzymes was inhibited when the pesticide Beltanol was added to the growth medium at a concentration of 0.25 ml/L. This suggests that the pesticide can kill the cell walls of fungi to prevent the production of fungal enzymes. This is in line with a study by [44] that demonstrated how certain pesticides caused the production of degradative enzymes like cellulase and pectinase to be inhibited in fungi. Fungicides work by destroying the fungal cell wall and cytoplasmic membrane, thereby destroying degradation enzymes [45]. Through the virulence of pathogens, including fungi, extracellular enzymes are crucial in the infection process [46].

Table 5. Shows the extracellular enzyme activity of the studied fungi based on the Enzyme Activity Index (EAI) with the addition of Beltanol pesticide treatment at a concentration of 0.25 ml/L.

Enzyme	Fungi	Colony diameter (cm)	Halo diameter (cm)	Enzyme activity	Assessment of activity	Enzyme activity with Beltanol pesticide treatment
Cellulase	*Aspergillus flavus*	2.00	2.50	1.25	+	_
	A . niger	2.00	2.25	1.12	+	_
	Cladosporium oxysporum	1.20	1.8	1.50	+ +	_
	Penicillium digitatum	1.50	3.50	2.33	+ + +	_
	P .expansum	0.73	1.03	1.41	+ +	_
lipase	*Aspergillus flavus*	3.5	_	_	+ + +	_
	A . niger	3.11	_	_	+ + +	_
	Cladosporium oxysporum	1.22	_	_	+ +	_
	Penicillium digitatum	1.63	_	_	+ +	_
	P .expansum	1.55	_	_	+ +	_
protease	*Aspergillus flavus*	3.88	4.91	1.26	+ +	_
	A . niger	3.25	4.01	1.23	+	_
	Cladosporium oxysporum	1.31	1.92	1.46	+ +	_
	Penicillium digitatum	1.50	2.02	1.34	+ +	_
	P .expansum	1.73	3.21	1.85	+ + +	_

+++ High activity (1.75 to 2.0 cm).
++ Moderate activity (1.25 to 1.75 cm).
+ Low activity (1.10 to 1.25 cm).
- Neglect or absence (1.00 to 1.10 cm).

Conclusion

Tissue culture is one of most promising procedure in plant propagation, and producing a true-to-type Plants, but facing several obstacles, including fungal Contamination. Our Study revealed the isolation of many different fungal species. most importantly, *Aspergillus, flavus A. niger, Cladosporium oxysporum, Penicillium expansum and Penicilliam* sp. from Banana cultured tissues CV. Grand 9. Additionally, the effectiveness of Beltanol fungicide has been proved among other fungicides by inhibiting the radial growth of fungal contaminants, completely and prevented of them from Producing any extracellular enzymes (Cellulase, Protease and Lipase). The phytotoxicity on Banana tissues

revealed no toxic effect of Beltanol on examined tissues. Our results recommend the application of Beltanol fungicide for Banana tissue cultures as a Protection measure for control fungal contamination.

References

[1] Dotto, J., Matemu, A. O., & Ndakidemi, P. A. (2019). Nutrient composition and selected physicochemical properties of fifteen Mchare cooking bananas: A study conducted in northern Tanzania. Scientific African, 6, e00150.
[2] Ashokkumar, K., Elayabalan, S., Shobana, V. G., Sivakumar, P., & Pandiyan, M. (2018). Nutritional value of cultivars of Banana (Musa spp.) and its future prospects. Journal of Pharmacognosy and Phytochemistry, 7(3), 2972-2977.
[3] Ireri, M. (2018). Banana production. Horticultural News.
[4] Bridgen, M. P., Van Houtven, W., & Eeckhaut, T. (2018). Plant tissue culture techniques for breeding. Ornamental Crops, 127-144.
[5] Ahmed, A.N. and Abass. M.H. (2022a). Disease Note: First Report of Cladosporium ramotenellum Schub., Zalar, Crous & Braun, 2007 (Fungi: Dothideomycetes) as a Potential Contaminant of Date Palm Tissue Culture. Basrah J. Agric. Sci. 35(2): 373-375.
[6] Kane, M. (2003). Bacterial and fungal indexing of tissue cultures. Journal of Allergy and Immunology, 94, 393-400.
[7] Ahmed, A.N. and Abass. M.H. (2022b). Phenotypic and molecular identification of fungal contaminants of date palm (Phoenix dactylifera L.) tissue culture in Iraq. Neuro Quantology. 20(7): 664-669.
[8] Ali, H., Nirmala, C., & Sharma, M. L. (2009). Control of in vitro contamination in bamboos. Plant Cell Biotechnology and Molecular Biology, 10(3/4), 119-124.
[9] Cobrado, J. S., & Fernandez, A. M. (2016). Common fungi contamination affecting tissue-cultured abaca (Musa textiles Nee) during initial stage of micropropagation. Asian Research Journal of Agriculture, 1(2), 1-7.
[10] Izarra, M. L., Panta, A. L., Maza, C. R., Zea, B. C., Cruzado, J., Gutarra, L. R., ... & Kreuze, J. F. (2020). Identification and control of latent bacteria in in vitro cultures of sweetpotato [Ipomoea batatas (L.) Lam]. Frontiers in Plant Science, 903.
[11] Ankur, V., Meena, B., & Harsh, N. S. K. (2014). Identification and bioassay of fungal contaminants observed during in vitro propagation of Saracaasoca (Roxb.) De Wilde. Biotechnology. International., 7(2), 35-42.
[12] Hof, H. (2001). Critical annotations to the use of azole antifungals for plant protection. Antimicrobial agents and chemotherapy, 45(11), 2987-2990.
[13] Bernardes, M. F. F., Pazin, M., Pereira, L. C., & Dorta, D. J. (2015). Impact of pesticides on environmental and human health. Toxicology studies-cells, drugs and environment, 195-233.
[14] Abdulmoohsin, R. G., Lahuf, A. A., Husain, Y. N. & Hameed, Z. L. (2019). Bioefficiency of some indigenous biocontrol agents against Rhizoctonia solani causing cowpea seed rot and preemergence damping-off. IOP Conf. Series: Earth and Environmental Science 388 012011.doi:10.1088/1755-1315/388/1/012011.
[15] Al-Kaby, A. M. S. (2004). The effect of some antibiotics and fungicides on the growth of embryogenic callus of date palm Phoenix dactylifera L. Basrah Journal for Date Palm Research. 3(1/2):97-110.
[16] Matrood, A.A.A. and A. Rhouma. (2021a). Efficacy of foliar fungicides on controlling early blight disease of eggplant, under laboratory and greenhouse conditions. Novel Research in Microbiology Journal, 5(3): 1283- 1293.
[17] Lahuf, A. A., Kareem, A. A., AL-Sweedi, T. M. and Alfarttoosi, H. A. (2019). Evaluation the potential of indigenous biocontrol agent Trichoderma harzianum and its interactive effect with nanosized ZnO particles against the sunflower damping-off pathogen, Rhizoctonia solani. IOP Conf. Series: Earth and Environmental Science 365: 012033. doi:10.1088/1755-1315/365/1/012033.
[18] Jasim, A. A., Mohammed, B. T. & Lahuf, A. A. (2019). Molecular and enzymatic properties of fungi isolated from historical manuscripts preserved at the Al-Hussein Holy Shrine. Biochem. Cell. Arch., vol. 19, no. 2, 2019.
[19] Matrood, A. A. A., and Rhouma,A. (2021b). Evaluating eco-friendly botanicals as alternatives to synthetic fungicides against the causal agent of early blight of Solanum melongena. Journal of Plant Diseases and Protection.128(6), 1517-1530.
[20] Lahuf, A.A., Kareem, A.A., Al-Sweedi, T.M., Alfarttoosi, H.A. (2019). Evaluation the potential of indigenous biocontrol agent Trichoderma harzianum and its interactive effect with nanosized ZnO

particles against the sunflower damping-off pathogen, Rhizoctonia solani. In IOP Conference Series: Earth and Environmental Science, 365
[21] Geiser, D. M., & LoBuglio, K. F. (2001). The monophyletic Plectomycetes: Ascosphaerales, Onygenales, Eurotiales. Systematics and Evolution: Part A, 201-219.
[22] Lahuf, A. A., Abdalmoohsin, R. A. G. & Alhusani, A. H., Al-Asadi, A. (2018). First report of leaf blight disease in lily (Lilium candidum) caused by Alternaria alternata in Iraq. Biopestic. Int. 14 (2): 123-126.
[23] Shaaban, Awwad and Nizar Mustafa Al-Mallah. 1993. Pesticides. Mosul University Press. 530 pages.
[24] Lahuf, A.A., Abdullah, K.M., Mohammadali, M.T. (2020). Assessment of the nanosized particles of ZnO and MgO and some cultivars in control of Alternaria solani causing tomato early blight. Ecol. Environ. Conserv. 26, 89–95.
[25] Al-Tememe, Z.A.M., Lahuf, A., Abdalmoohsin, R.G., Al-Amirry, A.T. (2019). Occurrence, identification, pathogenicity and control of Neoscytalidium dimidiatum fungus, the causal agent of sooty canker on Eucalyptus camaldulensis in Kerbala Province of Iraq. 2019. Plant Arch., 19, 31–38.
[26] Reese,E.T. and Mandels,M.(1963). Enzymic hydrolysis of cellulose and its derivatives .In Cellulose. 139 – 143pp.
[27] Sierra,G.(1957). A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek, 23(1):15-22.
[28] Cowan,J.T.(1986). mamual for the identification of medical bacteria .2nd camb. Umiv.Press London :146-156.
[29] Abass, M. H. (2013). Microbial contaminants of date palm (Phoenix dactylifera L.) in Iraqi tissue culture laboratories. Emirate Journal of Food and Agriculture, 25(11), 875-882.
[30] Al-Mayahi, A. M., Ahmed, A. N., & Al-Khalifa, A. A. (2010). Isolation and identification of associated fungi with the micropropagation of five different date palm cultivars and the effect of Benlate fungicides in their control. Basra Journal of Date Palm Res, 9(2), 79-97.
[31] Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Cancer as a microevolutionary process. In Molecular Biology of the Cell. 4th edition. Garland Science.
[32] Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., & Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. Molecules, 20(5), 8856-8874.
[33] Corkley, I., Fraaije, B., & Hawkins, N. (2022). Fungicide resistance management: Maximizing the effective life of plant protection products. Plant Pathology, 71(1), 150-169.
[34] Sanchez C., Moore D., Robson, G., Trinci, T. (2020). 21st century miniguide to fungal biotechnology/ Una miniguía del siglo XXI para la biotecnología de hongos. Mexican Journal of Biotechnology, 5(1):11-42.
[35] Sharma A, Kumar V, Kumar R, Shahzad B, Thukral AK, Bhardwaj R.(2018a) Brassinosteroid-mediated pesticide detoxification in plants: A mini-review. Cog Food Agric. 4: doi.org/10.1080/23311932.2018.1436212.
[36] Shahzad B, Tanveer M, Che Z, Rehman A, Cheema SA, Sharma A, Song H, Rehman S, Zhaorong D.(2018) Role of 24-epibrassinolide (EBL) in mediating heavy metal and pesticide induced oxidative stress in plants: A review. Ecotoxicol Environ Saf. 147: 935-44.
[37] Hedlund J, Longo S, York R (2019) Agriculture, pesticide use, and economic development: a global examination (1990–2014). Rural Sociol 85:519–544.
[38] Cernohlavkova J, Jarkovsky J, Hofman J (2009) Effects of fungicidesmancozeb and dinocap on carbon and nitrogen mineralization insoils. Ecotoxicol Environ Saf 72:80–85.
[39] Bokhary,H.A. and Parrz,S.(1994). Extracellulae cellulose enzyme production by soil mycoflora in Saudi Arabia. King saud Univercity, 6(2): 137 -148.
[40] Jumah, Iman Muhammad& al-Sadun, Abd Allah Hammud& al-Dusari, Mustafa Abd al-Wahhab Najm. (2020). Enzymatic activity of some fungi isolated from submerged plant parts in aquatic habitats southern Iraq. Marsh Bulletin،Vol. 15, no. 2, pp.83-91.
[41] Ellaiah P, Adinarayana K, Bhavani Y, Padmaja P. and Srinivasulu B. (2002). Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated Aspergillus species. Process Biochem, 38: 615- 620.
[42] Anitha TS, Palanivelu P (2013) Purification and characterization of an extracellular keratinolytic protease from a new isolate of Aspergillus parasiticus. Protein Expr Purif 88:214-220.
[43] Sunitha,V.H., NirmalaDevi,D. and Srinivas,C.(2013). Extracellular Enzymatic Activity of Endophytic Fungal Strains Isolated from Medicinal Plants. World Journal of Agricultural Sciences.,9(1):1-9.

[44] Raju, E. V. N., & Divakar, G. (2013). Screening and isolation of Pectinase producing bacteria from various regions in Bangalore. International Journal of Research in Pharmaceutical and Biomedical Sciences, 4(1), 151-154.
[45] Masomi, F., & Hassanshahian, M. (2016). Antimicrobial activity of five medicinal plants on Candida albicans. Iranian Journal of Toxicology, 10(6), 39-43.
[46] Madhu, S. N., Pal, A. K., & Gajjar, D. U. (2019). Extracellular proteases from keratitis causing Fusarium, Aspergillus and Dematiaceous species. Trends Ophthalmol Open Access J, 2(2), 1-9.