In vitro Propagation of *Mentha pulegium* L. and Testing, The Antifungal Activity of Stem Nodes Callus and Shoots Extracts Against *Acremonium strictum* L

Reem Ebraheem[*1], Daniel Al-awad[*2], Maysa Yaziji[*3]

[*1]*Researcher, Department of Plant Biology, Faculty of Science, Tishreen University, Lattakia, Syria.*
[*2]*Professor of Plant Propagation, Department of Plant Biology, Faculty of Science, Tishreen University, Lattakia, Syria.*
[*3]*Professor of Mycology, Department of Plant Biology, Faculty of Science, Tishreen University, Lattakia, Syria.*

Abstract:

This research aims to propagate buds of Mentha pulegium and forming callus, then determine the antifungal activity of ethanol, methanol for both stem node callus extract, and shoots extract M.pulegium against Acremonium strictum. The stem nodes segments of M. pulegium were cut into 0.5-1 cm in length and cultured on Murashige and Skoog (MS) solid medium supplemented with 3% sucrose, and different concentration of benzyl amino purine (BAP) was added either alone to propagate buds or in combination with 2,4-Dichlorophenoxyacetic acid (2;4-D) to form a callus. The MS was gelled with 8% agar.

Antifungal activity was performed in Petri dishes (poisoned food). The maximum number of callus (98.33±1.96%) was obtained on MS medium supplemented with 0.5mg/l BAP in combination with 1mg/l 2,4-D, and the maximum number of shoots (20±0.88) was obtained on MS medium supplemented with 1mg/l of BAP.

The results showed a higher efficiency of ethanol extracts than those of methanol. Ethanol and methanol extracts of callus were generally better than shoots extracts. MIC (Minimum Inhibition Concentration) value of the callus extract was 0.02 g / ml when using ethanol or methanol as an organic solvent, but the MIC value of the shoots extract was 0.03g/ml when using ethanol or methanol as an organic solvent.

Keywords*: Mentha pulegium*, shoot, callus, *Acremonium strictum*, Antifungal activity**.**

I. INTRODUCTION

Plant tissue culture technology is used to overcome many difficulties in propagating many species of plants, mostly medicinal and economic plants [1], where the genetic diversity of many plants is threatened as a result of their constant collection to take the advantages of their products medically and pharmacologically [2]. Medicinal plants have become the focus of intense study invalidating their traditional uses by determining their actual pharmacological effects[3]. Pharmaceutical companies have spent a lot of time and money developing natural products extracted from plants to produce remedies less cost and more effective, affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources [4]. The use of plants for healing purposes forms the origin of modern medicine, where many drugs originated from herbal sources [5]. Contrary to synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases ([4], [6]). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority has not been adequately evaluated [7]. The *genus Mentha* includes 25 to 30 species that grow in the temperate regions of Europe, Asia, Australia, and South Africa[10],[1]. *M. pulegium* is an important, medicinal, economical, wild plant that belongs to the Lamiaceae family and genus *Mentha*. It is also a short-lived perennial mint and one of the native species of Europe, North Africa, Asia Minor, and the Middle East [8].

M. pulegium has high medical importance, which is used in the treatment of gastrointestinal pain, spongy spleen, expectorant for sputum, purifies the chest, headache, and brain tonic. It is also used in the preparation of oral disinfectants. It contains an essential aromatic oil called Polygon, which is used in the preparation of many pharmaceutical products [9]. All parts of *M. pulegium* can be used except roots.

Plant regeneration via *in vitro* culture biotechnologies is regarded as a key factor for the realization of mass propagation of elite and standardized plants in the optimized environmental conditions ([10],[11]).

A. sitrticum belongs to Hypocreales，Hypocreaceae. The genus *Acremonium* contains about 150 species,

most of them are saprobes in soil and pathogens of plants, insects, and other fungi. Some species are considered opportunists of humans and other mammals. ([12], [13]), Infections in humans typically develop following traumatic inoculation of the fungus.

A. Strictum is a cause of the keratitis also and has a more recently significant role in onychomycosis. ([14], [15]).

Crude extracts of callus and *in vitro* plants are used in many bioactive experiments as antimicrobial or antifungal, which have also been used as an alternative to crude extracts of field plants because plant tissue culture can be applied at any time of the year. It can also produce new compounds in calli, which may not be existed in the original plant ([16], [17]).

M.pulegium spreads in many regions of the Syrian coast. It can be found on the sides of rivers, ponds, and inside the moist sites.

Wild plants are exposed to many bacterial, viral, and parasitic, affecting their crops. Also, they are collected randomly for their multiple uses so that they may be exposed to the danger of disappearance and due to the serious side effects of chemical antifungal.

This research is dedicated to propagate *M. Pulegium in vitro* and find safe, natural compounds as an alternative material to wild *M. Pulegium*.

II. Materials and Methods*:*

A. Plant Samplings

The stem nodes were cultured on the MS nutrient medium supplemented with various concentrations of the cytokinin BAP. Then the formed shoots were taken from the best MS medium supplemented with 0.5 mg /L of BAP to be propagated after that; plantlets were grown on the MS medium without hormone. The stem nodes were cultured in MS medium with various concentrations of auxin 2,4-D in combination with BAP to form a callus.

All cultured incubated at 25C^{o} with 16 hours photoperiod alternative with 8 hours darkness.

In vitro shoots were collected from the plantlets after two months. Callus was obtained from the best MS medium supplemented with 1 mg /L of auxin 2,4-D combined with 0.5 mg / L of cytokinin BAP.

In vivo, shoots of *M. pulegium* were collected from the Btara river in Jabla (Lattakia) before flowering from June to August 2019. In the lab, samples were washed by running tap water many times; then they were dried in the open air and in a shadow place. The samples were placed in an oven at 40 $^{\circ}$C until a constant weight was attained. Then, they were finely ground using an electric grinder, and the powder was kept in tightly sealed containers in a fridge until use.

B. Isolated Acremonium strictum

A. strictum was isolated from the case of onychomycosis. The isolated fungi were cultured on a sterilized nutrient medium of potato dextrose agar PDA, Then they were incubated at 25°C for seven days. Grown fungi species were isolated and purified.

The classification was based on morphological and microscopic criteria according to taxonomy references [18]. The isolated fungus was stored in PD. Tubes to be enriched a week again before making new cultures.

C. Preparing Organic Extracts

45 g of each powder of *in vivo* shoots and fresh *in vitro* shoots were placed in 500ml flasks in which 300ml of ethanol or methanol was added. 10g of fresh callus was placed in 25ml flasks in which 10ml of ethanol or methanol was added.

The mixture was vigorously stirred for half an hour using an electric agitator. The flasks were wrapped with aluminum foil and left in darkness for 20 days with keeping shaking from time to time over the mentioned period.

The extracts were filtered using Whatman paper NO.1 and porcelain filter. The process was repeated three times until the plant material was completely separated from the organic solvent. In order to thoroughly separate the plant extracts, the plant residuals were separated from the extracts, and then they were concentrated using a rotary evaporator at 40°C until cohesive, soft, and dough-like extracts were obtained. Then, they were kept in tightly sealed containers at 4°C until use.

4. Antifungal Assay:

The antifungal assay was done using the petri dish method according to reference [18] with some suitable modification. The crude extracts were diluted in water and added to P.D.A. contained in flasks at concentrations of (0.005, 0.01 ,0.015, 0.02, 0.03) g/ml. Then the media were poured in 9cm Petri dishes. After that, a 5mm^3 cube was taken from the edge of each 7 days old colony of the studied fungus and placed in the middle of Petri dishes. Then they were incubated at 25°C for 7 days. Control Petri dishes contained free-extract PD. An on which the study fungus was planted.

Antifungal assay for each concentration of the extract was performed in triplicates, and the culture plates of the fungal colonies were measured. Then the inhibition percentage was calculated.

III. Results and Discussion

A. Shoots formation and callus induction

Shoots formation and Callus induction were observed on the stem nodes, which were cultured on the MS medium supplemented with the various BAP concentrations alone or in combination with 2,4-D. Young cultured explants on the MS medium supplemented with BAP showed a good number of shoots. The maximum number of shoots formation was obtained on MS medium supplemented with 1 mg/L of BAP (20±0.88 shoots). (Table I, Fig.1)

A high percentage of callus showed on the medium supplemented with 1mg/L of 2,4-D combined with 0.5 or 0.1 mg/l of BAP.

The MS medium supplemented with 1mg/L of 2,4-D in combination with 0.5mg/L of BAP showed 98.33±1.96 percentage of callus induction, and the MS medium supplemented with 1mg/L of 2,4-D in combination with 0.1mg/L of BAP showed 98±2.1 percentage (Table I, Fig.2).

The influence of 2,4-D and BAP on shoots formation and callus induction was observed by a number of tissue culture ([19], [20]). In our study, the best percentage of callus induction was between 98-98.33% when using 0.1mg/L to 0.5 mg/L of cytokinin BAP. Our result was directly consonance with the [21] observation on *Mentha spicata;* that the callus induction was 72.2% in 0.5mg/L of BAP and increased to 84.6% in 1mg/L of BAP in combination with 2mg/L of 2,4-D.

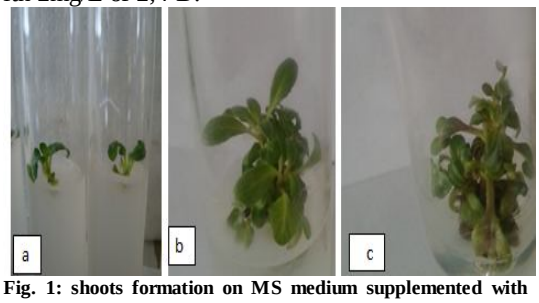

Fig. 1: shoots formation on MS medium supplemented with 1 mg/l of BAP (a: stem nodes, b, c: shoots formation)

Fig.2: callus formation on MS medium supplemented with 1mg/l of 2,4-D in combination with 0.5 mg/l of BAP.

Table I: Influence of 2.4D and BAP on callus induction and shoots formation from the stem nods segments of *Mentha pulegium*.

MS medium + plant growth regulator (mg/l)		Mean percentage of callus induction ± SE.	Mean number of shoots formation ± SE
2,4-D	BAP		
0	0	0	3±0.55
0	0.5	-	12±0.33
0	1	-	20±0.88
0	2	-	14±66
0	3	-	10±0.55
0	4		9±0.66
1	0.1	98±2.1	-
3	0.1	50.33±1.24	-
5	0.1	26.33±1.24	-
7	0.1	18.66±1.24	-
1	0.5	98.33±1.96	-
3	0.5	64±0.81	-
5	0.5	51.66±1.24	-
7	0.5	19±0.81	-

B. Isolating and Identifying of A .strictum:

A. strictum grown readily at 25°C on PDA, the mycelium appeared of approximately 50mm in size in 7 days. Colonies are flat, with a smooth, wet, velvety texture, sometimes resembling thin cottony mounds, and the color of mycelia ranges widely from light pink to orange Fig.1(A). The filaments are bound together into ropes; conidia grow as wet clusters or dry chains Fig.3 (B, D). The conidia appear ellipsoidal to cylindrical Fig.1(C).

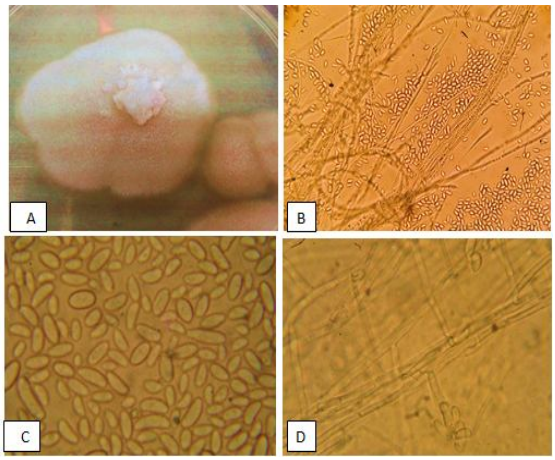

Fig.3: *Acremonium strictum*
A: colony, B, D: filaments, C: conidia

C. Antifungal assay:

The results of antifungal screening tests of stem nodes extracts and *in vivo* or *in vitro* shoot extracts of *M. pulegium* in the different solvent; ethanol, methanol against *A. strictum* are depicted in Table II, III. It was obvious from the findings that ethanol and methanol extracts of *M.pulegium* revealed antifungal activity against *A. strictum* in different rates due to the source of plant material, the type of solvent, and the extract concentration.

All extracts exhibited antifungal activity at different concentrations, as shown in Table II. Several studies have shown that the extract of an aromatic plant, such as *Mentha arvensis*, *Rosemary*, and *Thymus vulgaris* showed good antifungal activity against a number of pathogenic fungi ([15], [22]).

Antifungal potential depends on the source of the plant part, where EtOH extract of callus was the most effective in comparison to EtOH extracts of *in vitro* and *in vivo* shoots. So the diameter of *A. strictum* colony was 0cm at a concentration of 0.02 g/ml, with an inhibitory rate of 100 %, while at the same extract concentration, it was 0.27±0.33cm, and the rate of inhibition was 94.16% when treated with EtOH extract of *in vivo* shoots, and it was 0.46±0.31cm, and the rate of inhibition was 89.91% when treated with EtOH extract of *in vitro* shoots.. (Fig4, Fig5, Tab II).

Table II: Colony Diameters (cm) of *A. strictum* at Different Concentrations of EtOH Extracts of *M. pulegium* on the 7th Days Incubation.

Extracts Concentration g/ml	Plant parts		
	callus	*In vitro* shoots	*In vivo* shoots
Control=0	4.6±0.07	4.56± 0.11	4.63±0.11
0.005	2.5±0.18	3.16±0.15	3.16±0.11
0.01	1.46±0.11	2±0.07	1.3±0.07
0.015	0.66± 0.41	1.23±0.17	0.73±0.45
0.02	0	0.46±0.31	0.27±0.33
0.03	0	0	0

Table III: Colony Diameters (cm) of *A. strictum* at Different Concentrations of MeOH Extracts of *M. pulegiu*m on the 7th Days Incubation.

Extracts Concentration g/ml	Plant parts		
	callus	*In vitro* shoots	*In vivo* shoots
Control=0	4.6± 0.12	4.6±0.12	4.6±0.12
0.005	3.16±0.11	3.56±0.15	3.6±0.37
0.01	1.3±0.07	1.86±0.11	2.3±0.35
0.015	0.86±0.14	0.9±0.07	1.03±0.11
0.02	0	0.66±0.11	0.6±0.32
0.03	0	0	0

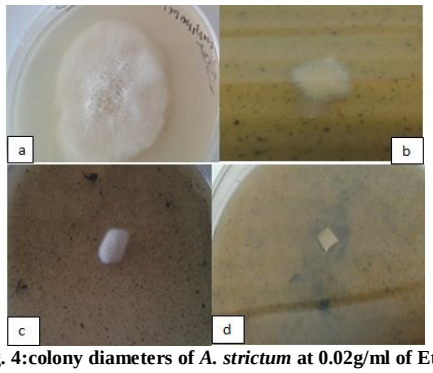

Fig. 4:colony diameters of *A. strictum* at 0.02g/ml of EtOH extracts (a: control, b: EtOH *in vitro* shoots extract,c: EtOH *in vivo* shoots extract, d: EtOH callus extract).

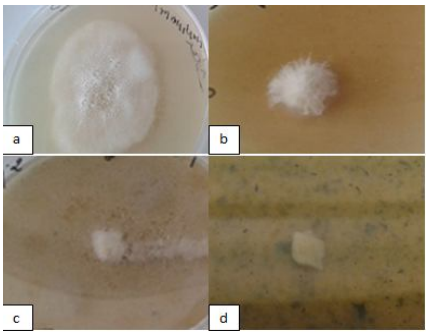

Fig.6:colony diameters of *A. strictum* at 0.02g/ml of MeOH extracts (a: control, b: MeOH in vivo shoots extract,c: MeOH in vitro shoots extract d: MeOH callus extract).

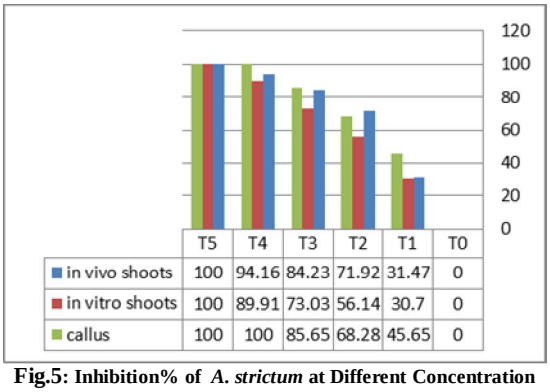

Fig.5: Inhibition% of *A. strictum* at Different Concentration of *M. pulegium* EtOH Extracts. (T0= 0 g/ml, T1=0.01g/ml, T2= 0.015 g/ml, T3= 0.02g/ml, T4= 0.03 g/ml, T5= 0.04g/ml.

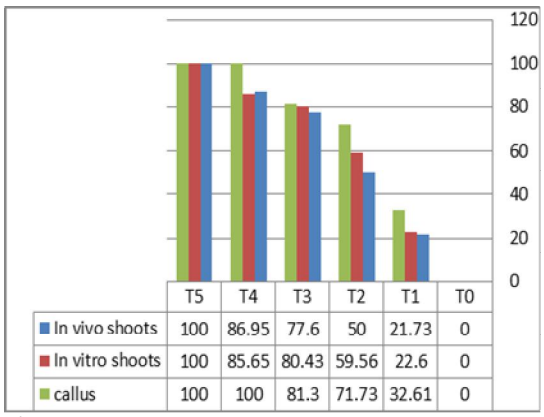

Fig.7: Inhibition% of *A. strictum* at Different Concentration of *M. pulegium* MeOH Extracts.
(T0= 0 g/ml, T1=0.005g/ml, T2= 0.01 g/ml, T3= 0.015g/ml, T4= 0.02 g/ml, T5= 0.03g/ml.)

Table III shows the average growth rates of *A. strictum* after 7 days of incubation at 25 ° C in different concentrations of MeOH extracts. All concentrations of extracts have affected the growth of *A. strictum*, and the stem nodes callus extracts were the most effective in comparison to the *in vitro* or *in vivo* shoots extracts. So the diameter of *A. strictum* was 0cm at a concentration of 0.02 g/ml, with an inhibitory rate of 100% when treated with callus extracts, while at the same concentration, it was 0.66±0.11cm, and the rate of inhibition was 86.56% when treated with MeOH in vitro shoots extracts, and diameter of the colony was 0.6±0.32cm, and the rate of inhibition zone was 86.95% when it was treated with MeOH *in vivo* shoots extracts (Tab III, Fig: 6, Fig7).

The MIC value of the ethanol or methanol callus extracts was at 0.02 g/ml and was at 0.03g/ml for the shoots extracts in ethanol or methanol solvent.

Our study was similar to several studies in which the extract of the fresh callus extracts of *M. arvensis or extracts of M. pulegium* showed good antimicrobial or antifungal activity against many species of pathogenic organisms [13] or those studies showed antifungal activity of aromatic plants against Acremonium sp. [23]. While this research is the first to describe the antifungal activity of callus and *in vitro* shoots extracts of *M. pulegium* against *A. strictum.*

Several studies indicated that the aromatic plants were a good source of potential material.

Phytochemical constituents such as tannins, saponins, flavonoids, alkaloids, and several other aromatic and regenerated potential compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects, and other herbivores ([24], [25]).

The efficacy of the callus extract may be due to containing an active substance, which is formed when the mutations occur in callus cell, which leads to producing proteins, which play an important role in stimulating the formation of metabolic compounds not present in the mother plant [26].

It has been found in Tab. II, III; Fig 5,7 that the effect of the callus extracts was better than the effect of *in vitro* and *in vivo* shoots extracts in most concentrations. Many studies have shown the effectiveness of the genus *Mentha* in different types against most microorganisms, including fungi.

This activity is because *Mentha* contains flavonoids and various types of essential oils, especially Menthon, Menthol, Menthe acetate. Also, *M.pulegium* contains the volatile Minton and polygon oils that have antifungal activity([21], [22]).

Antifungal activity of *M.pulegium* against *A. strictum* may be due to the use of raw extracts (a mixture of compounds) which contain a combination of active compounds rather than a specific substance. Recent research has indicated that the use of new raw compounds of tissue culture has a greater benefit in terms of the integrated effect on microorganisms.

The use of crude extracts, especially callus extract, reduces the resistance to these organisms, which occurs when resistant strains are found by the repetitive use of chemical pesticides or chemical drugs [27].

IV. Conclusion

1. The MS medium supplemented with 1mg/l of BAP was the best medium to propagate the buds of *M .pulegium.*
2. The MS medium supplemented with 1mg/L of 2,4-D in combination with 0.5 or 0.1 mg/L of BAP was the best to form the callus of *M. Pulegium.*
3. Ethanol extract of *M. pulegium* callus exhibits the highest inhibitory activation against *A. strictum.*
4. The study findings suggested to use *M. pulegium* crud extracts to control the spread of *A. strictum.*
5. The study findings suggested modifying the new compounds which are in *M. pulegium* callus extract to control the spread of *A. strictum.*

V. ACKNOWLEDGMENTS

The authors acknowledge the help of Tishreen University, college of science, and the Department of Botany.

REFERENCES

[1] Ivanova, M., Van Staden, J., "*Influence of gelling agent and cytokinins on the control of hyperhydricity in Aloe polyphylla. Plant Cell, Tissue, and Organ Culture (PCTOC)*" 104, 13-21, 2011.

[2] Anish, N., Dan, M., Bejoy, M., "*Conservation using in vitro progenies of the threatened ginger Boesenbergia pulcherrima (Wall.) Kuntze*". Int. J. Bot 4, 93-98, 2008.

[3] Johnson, M., Wesely, E., Kavitha, M., Uma, V., "*Antibacterial activity of leaves and inter-nodal callus extracts of Mentha arvensis L*". Asian Pacific journal of tropical medicine 4, 196-200, 2011.

[4] Doughari, J., "*Antimicrobial activity of Tamarindus indica Linn.*" Tropical Journal of Pharmaceutical Research 5, 597-603. 2006.

[5] Jazani, N., Ghasemnejad-Berenji, H., Sadegpoor, S., "*Antibacterial effects of Iranian Mentha pulegium essential oil on isolates of Klebsiella sp*". Pakistan Journal of Biological Sciences 12, 183, 2009.

[6] Maghrani, M., Zeggwagh, N.-A., Michel, J.-B., Eddouks, M., "*Antihypertensive effect of Lepidium sativum L. in spontaneously hypertensive rats*". Journal of Ethnopharmacology 100, 193-197, 2005.

[7] Balandrin MJ, Klocke JA. "*Medicinal, aromatic and industrial materials from plants*". Berlin: Bajaj Springer-Verlag, p.1-36. 1988.

[8] Dorman, H.D., Koşar, M., Kahlos, K., Holm, Y., Hiltunen, R., "*Antioxidant properties, an aqueous composition extracts from Mentha species, hybrids, varieties, and cultivars*". Journal of agricultural and food chemistry 51, 4563-4569, 2003.

[9] Sarikurkcu, C., Eryigit, F., Cengiz, M., Tepe, B., Cakir, A., Mete, E., "*Screening of the antioxidant activity of the essential oil and methanol extract of Mentha pulegium L. from Turkey*". Spectroscopy Letters 45, 352-358, 2012.

[10] Canter, P.H., Thomas, H., Ernst, E., "*Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology*". TRENDS in Biotechnology 23, 180-185, 2005.

[11] Zarki, K.B.L., Elmtili, N., "*Micro-propagation of Mentha pulegium L. through high-frequency shoots-tip and nodal explants culture Moroccan Journal of Biology N 8-9*". Département de Biologie, Faculté des Sciences, Av. de Sebta, Mhannech II, Tétouan, 93002 Morocco, 2012.

[12] Ebrahimi, L., Fotouhifar, K., "*Identification of some fungi accompanying the scab symptoms in Iran*". Mycologia Iranica 3, 25-37. 2016.

[13] Perdomo, H., Sutton, D., García, D., Fothergill, A., Cano, J., Gené, J., Summerbell, R., Rinaldi, M., Guarro, J., "*Spectrum of clinically relevant Acremonium species in the United States*." Journal of Clinical Microbiology 49, 243-256. 2011.

[14] Anadolu, R., Hilmioğlu, S., Oskay, T., e Boyvat, A., Peksari, Y., Gürgey, E., "*Indolent Acremonium strictum infection in an immunocompetent patient*". International journal of dermatology 40, 451-453, 2001.

[15] Erdogan, O., Celik, A., Zeybek, A., "*In vitro Antifungal activity of Mint, Thyme, Lavander Extracts and Essential Oils on Verticillium Dahliae Kleb*". Feb-Fresenius Environmental Bulletin, 48562016.

[16] Khafagi, I.K., "*Variation of callus induction and active metabolite accumulation in callus cultures of two varieties of Ricinus communis L*". Biotechnology 6, 193-201, 2007.

[17] Taylor, D.C., Falk, K.C., Palmer, C.D., Hammerlindl, J., Babic, V., Mietkiewska, E., Jadhav, A., Marillia, E.F., Francis, T., Hoffman, T., Brassica carinata – a new molecular farming platform for delivering bio - industrial oil feedstocks: case studies of genetic modifications to improve very-long-chain fatty acid and oil content in seeds. Biofuels, Bioproducts, and Biorefining 4, 538-561, 2010.

[18] Suárez-Jiménez, G.M., Cortez-Rocha, M.O., Rosas-Burgos, E.C., Burgos-Hernández, A., Plascencia-Jatomea, M., Cinco-Moroyoqui, F.J., Actividad antifúngica de extractos vegetales sobre Fusarium verticillioides (Sacc.) Nirenb. y producción de fumonisina B1. Revista mexicana de fitopatología 25, 134-142, 2007.

[19] Chakraborty, N., Banerjee, D., Ghosh, M., Pradhan, P., Gupta, N.S., Acharya, K., Banerjee, M. "*Influence of plant growth regulators on callus mediated regeneration and secondary metabolites synthesis in Withania somnifera (L.)*

Dunal." Physiology and Molecular Biology of Plants 19, 117-125, 2013.

[20] Sujana, P., Naidu, C., "*Indirect Plant Regeneration from Leaf Explants of Mentha piperita (L.) â€"An Important Multipurpose Medicinal Plant."* Journal of phytology 3. 2011.

[21] Samantaray, A.; SIAL, P.; KAR, M., "*Micro-propagation and biochemical analysis of Spear Mint (Mentha spicata)*". Indian Journal of Innovations and Developments,vol. 1, 489-493. 2012.

[22] Lorán, S., Segman, O., Rota, C., Conchello, P., Herrera, A., "*In vitro antifungal activity of several essential oils from aromatic plants of Aragón (NE, Spain)",* Science And Technology Against Microbial Pathogens: Research, Development, and Evaluation. World Scientific, pp. 99-103. 2011.

[23] Racowski, I., Foramiglio, V., Teodoro, J., Freire, V., "*Antifungal Activity of Infusions from Fresh Oregano, Laurel, and Rosemary Leaves and Their Commercial Essential Oils against Acremonium sp.*" Journal of Microbiology Research 6, 35-39. 2016.

[24] Ćirić, A., Vinterhalter, B., Šavikin-Fodulović, K., Soković, M., Vinterhalter, D., *"Chemical analysis and antimicrobial activity of methanol extracts of celandine (Chelidonium majus L.) plants growing in nature and cultured in vitro"*. Archives of Biological Sciences 60, 7-8. 2008.

[25] Marimuthu, J., Antonisamy, A., "*Somoclonal variation studies on Phyllanthus amarus Schum & Thonn"*. Iranian Journal of Biotechnology 5, 240-245. 2007.

[26] Loyola-Vargas, V.M., Plant cell culture protocols. 2006.

[27] Sinha, R., Chattopadhyay, S., "*Changes in the leaf proteome profile of Mentha arvensis in response to Alternaria alternata infection*." Journal of proteomics 74, 327-336, 2011

[28] Reem Ebraheem, Daniel Al-awad, MaysaYaziji, "*Antifungal Activity of Mentha pulegiumCrud Extracts Against Alternaria citri*" SSRG International Journal of Agriculture & Environmental Science 4.6 (2017): 54-57.