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Cichorium intybus Roots Extract: A New Culture Medium for Cultivation of *Aspergillus niger, Aspergillus terreus, Fusarium graminearum* and *Candida albicans*

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Abstract:

For the first time globally, the studying of using hot water extract of Cichorium intybus (chicory) as a new culture medium for fungi and yeast growth was done .It used for cultivation of: *Aspergillus niger ,Aspergillus terreus,Fusarium graminearum* and *Candida albicans*.

The present study clearly showes the possibility of using the chicory roots extract agar medium as a new culture medium for molds and yeasts.

The results showed avariation in the growth of fungi on chicory roots extracts agar medium compared with potato dextrose agar medium (PDA) in colony growth diameter mean ,colony color, sporulation and producing of different color dyes.

The sporulation for *Aspergillus niger* and *Aspergillus terreus* was heavy on chicory roots extract agar medium compared with PDA medium.

In addition,the prepared medium will be usefull for fungal toxomony,for *Aspergillus terreus* and *Fusariun graminearum*, dark yellow pigment of *Aspergillus terreus* on chicory medium while it was yellow pigment on PDA. *Fusarium gramiearum* produce purple color pigment on chicory medium while it was pink pigment on PDA,also chicory medium was good medium for cultivation of *Candida albicans* without producing pigments.

Key Words*: Chicory roots, hot water extract, fungi, yeast.*

دراسة امكانية استخدام المستخلص المائي الحار جذور نبات الهندباء كوسط زرعي جديد لنموالاعفان والخمائرمن نوع *Aspergillus niger, Aspergillus terreus, Fusarium graminearum* and *Candida albicans*

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الخلاصة :

لأول مرة عالميا تم دراسة امكانية استخدام المستخلص المائي الحار لجذور نبات الهندباء كوسط زرعي جديد لنموالاعفان والخمائر، حيث استخدم هذا الوسط لتنمية *Aspergillus niger, Aspergillus terreu ,Fusarium graminearum* and*Candida albicans.* بينت الدراسة الحالية امكانية استخدام الوسط الزرعي المحضر من جذور الهندباء كوسط جديد لنمو الاعفان والخمائر، حيث استخدم هذا الوسط لتنمية *Aspergillus niger, Aspergillus terreus ,Fusarium graminearum and Candida albicans.*

بينت الدراسة الحالية امكانية استخدام الوسط الزرعي المحضر من جذور الهندباء كوسط جديد لنمو الاعفان والخمائر، حيث اظهرت النتائج تباين في نمو الفطريات على وسط مستخلص جذور نبات الهندباء مقارنه مع الوسط التقليدي بطاطا دكستروز اكار (PDA) من حيث معدل نمو القطري للمستعمرة، لون المستعمرة، عملية تكوين السبورات فضلاً عن إنتاج صبغات لونية مختلفة، وفيما يخص عملية تكوين السبورات فقد كانت أسرع وأكثر على الوسط المحضرمن مستخلص جذور الهندباء مقارنة بالوسط التقليدي PDA بالنسبة للفطر *Aspergillus niger ,Aspergillus terreus* ويمكن عد الوسط المحضر وسطا تشخيصيا للفطر *Aspergillus terreus ,Fusarium graminearum* وذلك للتغاير اللوني في شدة تلون المستعمرة وانتاج صبغات ملونة فقد اعطى الوسط الزرعي المحضر لونا اصفر داكن عند تنمية الفطر *Aspergillus terreus* مقارنة مع الوسط التقليدي PDA الذي اعطى لونا اصفربينما تلون وسط مستخلص جذور الهندباء بالصبغة البنفسجية الداكنة ولون المستعمرة ابيض عند تنمية الفطر *Fusarium graminearum* مقارنة بلون المستعمرة الوردي مع انتاج صبغة وردية اللون على الوسط PDA، كما تمكنت الخميرة *Candida albicans* من النمو وبسهولة على الوسط المحضر دون تلونها او انتاج صبغات لونية.

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Inroduction:

Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. Culture media used in the laboratory for cultivation of microorganisms supply the nutrients required for growth and maintenance when a medium is being prepared for microbial growth, consideration must be given to the provision of carbon and energy source and other factors that are essential for the organisms [1].

A wide variety of culture media is employed by the microbiologist for the isolation, growth, maintenance of pure cultures and identification of microorganisms according to their biochemical and physiological properties [2].

Many types of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, PH, temperature, light, water, availability and surrounding atmospheric gas mixture [3,4].

Potato Dextrose Agar (PDA) is commonly used for the isolation and growth of a wide range of fungi in laboratories [5,6] and its compositions are well defined [7].

In recent years some researchers concentrated to screen alternative culture media from locally available materials[8,9, 10].

Different media for the growth and isolation of organisms have been reported from different substrates.

Plant materials have been used to recover both fungi and bacteria from different sample sources such as ground nut, Sorghum extracts, local food stuff waste, Cassava whey, beans and pigeon pea, African oil bean [11].

Common chicory (*Cichorium intybus*) is a bushy perennial herb with blue or lavender flowers.

It grows as a wild plant on road sides in its native Europe and in North America, where it has become naturalized .it is grown for its leaves, or for the roots, which are backed, ground and used as a coffee substitute and additive.

Fresh chicory roots typically contains by dry weight 68% inulin,14% sucrose, 5% cellulose, 6% protein,4% ash and 3% other compounds.

Dried chicory contains by weight, approximately 98% inulin and 2% other compounds[12]. The present study is the first study to determine the ability of medium prepared from hot water extract of chicory roots to use as alternative medium for cultivation of three molds, *Aspergillus niger,Aspergillus terreus, Fusarium graminearum* and yeast *Candida albicans* .

Materials and methods:

Test microorganism:

in this study ,*Candida albicans, Aspergillus niger, Aspergillus terreus, Fusarium graminearum* were tested. These fungi were obtained from laboratories of Biology department, college of Science, University of Al-Mustansiryiah.

Prior to the study above yeast and fungi were subcultured on Potato Dextrose Agar medium (PDA).

Collection of plant:

Roots of *Cichorium intybus* (chicory) used in this study was collected and confirmed by National Herbarium of Iraq (Abu Garib).

Preparation of extract:

The dried roots were finally powdered using electric blender and extracted with hot water according to harborn, 1973 [13].

With some modification, roots powder (50gm) was put in 1L flask and distilled water (250 ml) was added, the mixture were heated in 100^{o}C for 30 minutes. This was then allowed to cool and the extract was filtered using sterile muslin, then it was filtered by using what ma filter paper. After filtration, the extract

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was sterilized by using millipore filter (0.22μm).

The sterile extract was placed in a sterile container and preserved in refrigator (4^{o}C) until used for preparation alternative culture medium. The PH of extract was 6.

Preparation of medium:

Chicory roots extract medium was prepared as a solid medium by adding sterile agar agar to the extract; this was done by dissolving 2 gm of agar agar in 20 ml distilled water and sterilized by using autoclave, then left to cool at 45^{o}C to add 80 ml of sterile chicory root extract .Approximately 20 ml of the sterilized chicory roots extract agar medium was placed in each of Petri plates

Microorganism's inoculation:

The test microorganisms inactively growing pure cultures were taken and 5mm discs of each fungus obtained from pure cultures were transferred at the centre of sterile Petri dishes (in triplicates) containing different growth media PDA and chicory extract agar. The Petri dishes were then incubation for 6 days at 28^{o}C.

After incubation period, the fungi were detected, and the diameters of growth were measured in chicory extract agar medium and compared with PDA.

Results:

In this study, attempt to improve a new growth medium prepared from chicory roots extract for yeast and molds instead of their conventional media. The results showed maximum mycelial growth on chicory roots extract agar after 6 days of incubation period (Table-1) , while the mean value of *Aspergillus terreus* (45mm), *Aspergillus niger* (90mm) and *fusarium graminearum* (47mm) and showed maxium growth on PDA medium as a compared medium, mean of value of *Aspergillus niger* (90mm), *Aspergillus terreus* (60mm) and *Fusarium graminearum* (41mm) while growth rate of yeast *Candida albicans* was weaker growth in chicory roots extract agar (16mm) than on PDA medium (25mm).

In the present study also showed variations in color of colony and sporulation; Aspergillus niger was black color in both media, *Aspergillus terreus* was dark yellow conidia in chicory roots extract agar medium and light yellow conidia at centre in PDA medium ,while *Fusarium graminearum* white condia at centre with dark violet pigement in chicory roots extract agar , white conidia to pink at centre on reverse side in PDA medium . On the other hands, sporulation of three molds on chicory roots extract agar was more heavily than on PDA medium, specially *Aspergillus niger* , *Aspergillus terreus* revealed heavy growth conidial production after 6 days of incubation period., whereas comparatively moderate fungal sporulation was observed in PDA medium. (Figure-1).

Table-1: Mycelia growth, colony characters and sporulation pattern of fungal isolates on two different culture media (chicory roots extract agar medium &PDA).

Fungi	Media type	Colony Diam. (mm)	Conidia colour	pigment	Sporulation
Aspergillus niger	PDA Chicory root extract medium	90± 0.0 90± 0.0	Black Black	None None	Moderate Heavy
Aspergillus terreus	PDA Chicory root extract medium	60± 0.02 45± 0.01	White to light Yellow Dark yellow	Yellow Dark yellow	Moderate Heavy
Fusarium graminearum	PDA Chicory root extract medium	41± 0.05 47 ± 0.06	White to Pink White	Pink Dark violet	Moderate Moderate
Candida albicans	PDA Chicory root extract medium	25± 0.01 16± 0.02	Creamish white Creamish white	None None	Moderate Poor

Data represents mean of three replicates ±SE

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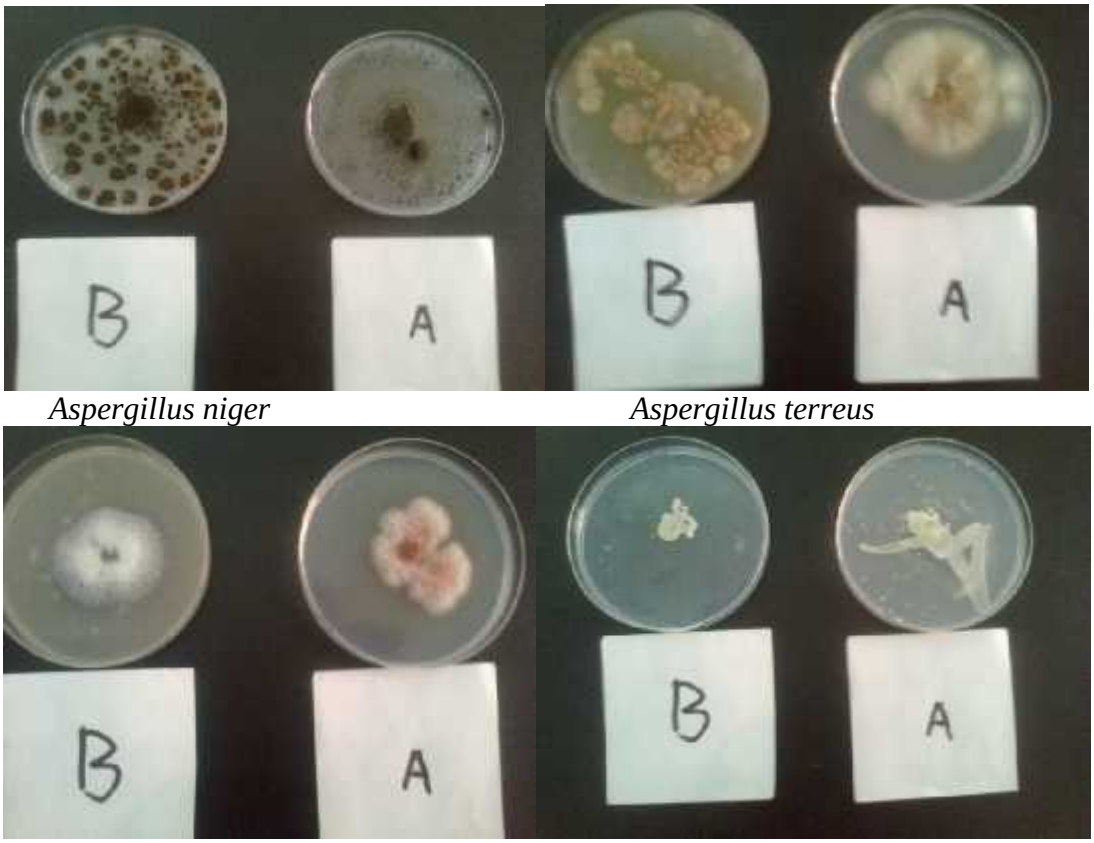

Aspergillus niger *Aspergillus terreus*

Fusarium graminearum *Candida albicans*

Figure-1: Colony, color and sporulation patterns of fungi on two different culture media: A- PDA culture medium: B- chicory roots extract agar medium.

Discussion:

The objective of this research was to develop an alternative and practical medium for the growth of Candida albicans, Aspergillus niger, Aspergillus terreus, Fusarium graminearum, the results presented in this paper clearly indicated that chicory roots extract medium can be used for the growth of above micro-organisms.

Complex media are rich in nutrients; they contain water soluble extracts of plants or animal tissue. Usually sugar or glucose is added to serve as main and energy source.

The combination of extracts and sugars creates a medium which is rich in minerals and organic nutrients [13].Two representative vegetable-based tryptic soy formulations were used to culture a range of bacteria and fungi [14], then the growth characteristics of them were compared with each other.

All the representative of microorganisms grew well on the vegetable based media and the media provided suitable recoveries of the organisms following simulated storage. Types of culture media and their chemical compositions significantly affected the growth rate and conidial production[15], hence this medium chicory roots extract agar is useful for fungal isolation and identification.

The fungal systematics is still based mainly on morphological criteria as observable characteristics.

Moreover, Fungi are recognized and identified basically by their phenotypes [16].

The variations in color of colony, especially among Aspergillus terreus and Fusarium graminearum, are one of the main criteria used widely for their identification and taxonomic placement[17]. Chicory roots contain inulin, sucrose, cellulose, protein[12] also contain elements

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like Ca, Na, K, P, Mg[18], and these contents make the chicory roots extract medium as a suitable medium to cultivate fungi specially molds. Further studies regarding the nature and chemical composition of this media and cultivation of other types of fungi are needed to enrich the value of this finding. Comparing with the performance on conventional bacteriological and mycological media, the chicory roots extract medium is found to be suitable and cheap medium material for the isolation and cultivation of fungi.

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