Inhibitory effect of some medicinal plant in Al-Jabal Al-Akhdar region against some postharvest fungi

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الفاعلية التثبيطية لبعض مستخلصات نباتات طبية في الجبل الأخضر ضد فطريات ما بعد الحصاد

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الملخص:

تهدف هذه الدراسة إلى تقييم الفعالية التثبيطية لبعض مستخلصات النباتات الطبية (ماء– ميثان– هكسان) من نبات اللوسينيا *Leucaena leucocephal* والعجرم *Anabasis aphylla* والرينش *Arum cyreniacum* والقطف *Atriplex halimus* والشيح *Artemisia annua* والعلندا *Ephedra alta* والحريق *Urtica urens* بتركيز 10% ضد نمو فطريات *Asperagillus niger*، *Penicillium italicum* و *Rhizopus stolonifer.*، كانت المستخلصات المائية لنبات العلندا *E. alta* ونبات اللوسينيا *L. leucocephala* ونبات الحريق *U. urens* هي الأكثر فعالية كمضاد للفطريات من المستخلصات الأخرى، كما أشارت النتائج إلى أن المستخلصات المائية والميثانولية أعطت كفاءة أكبر من مستخلصات الهكسان وكان *Asperagillus niger* أكثر الفطريات تأثراً بجميع المستخلصات المختبرة. وأظهرت بيانات هذه الدراسة إمكانية استخدام تلك المستخلصات كمبيدات فطرية صديقة للبيئة.

الكلمات المفتاحية: *تثبيط – نباتات طبية – فطريات ما بعد الحصاد.*

Abstract:

The present study aimed to evaluate the Inhibitory effect of some medicinal plants (water, methanol and hexanol) extracts such as *Leucaena leucocephala* (Jumby),*Anabasis aphylla* (,haze shrub), *Arum cyreniacum* (Cyrenaicum Calla Lil), *Atriplex halimus* (saltbush), *Artemisia annua* (sweet wormwood), *Ephedra alta* (alenda) and *Urtica urens*(annual nettle) at 10% concentraction against mycelial growth of *Asperagillus niger*, *Penicillium italicum* and *Rhizopus stolonifera*. The aqueous extracts of *E. alta*, *L. leucocephala* and *U. urens* were the most effective as antifungal than other extracts, also, the results indicated that the aqueous and methanol extracts gave more efficiency than hexane extracts and the A*speragillus niger* was the most affected fungus for all extracts tested. Data in this study showed the potential of using that extracts as environmentally friendly fungicides.

Keywords: *Inhibition, Medicinal plants, Postharvest fungi.*

Introduction:

Postharvest fungal infection of fruits and vegetables mainly caused by of *Asperagillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Botrytis* and *Rhizopus* (Saleh and Tahani, 2019) which are ubiquitous in nature and surving within wide range of temperature, humidity and PH (Martin *et al*., 2022). Moreover, fungal growth on fruits vegetables may lead to production of toxic compounds (Mycotoxins). Food and Agriculture Organization (FAO) documented about 25% of the world's agriculture commodities are contaminated with mycotoxins capable of causing diseases and death in human and animals (Bazioliet *et al*., 2019). Currently the postharvest diseases controlled by chemicals fungicides. But, due to development of microbial resistance towards fungicides and pollute environment the need to search for alternatives effective methods ecologically friendly for agricultural applications cannot be underestimated. The plant kingdom represents an enormous reservoir of phytochemical with various chemical materials and protective/–disease preventive properties such as alkaloids, steroids, flavonoids, terpenoids, tannis and many others (Abd-Ellatif *et al*.,2011). Medicinal plants in Libya have been part of the country's natural and cultural heritage for thousands of years. Currently, Eastern region of Libya is home to 179 different species, belonging to 166 genera and 72 families (El-Mokasabi *et al*., 2018)

During a random screening to investigate the biological activity of a range of medicinal plants species common in the study area, Among these plants *Leuceana leucocephala* belongs to the Fabaceae of the Leucaena genus. *L. leucocephala*, is a small fast growing small tree native to Southern Mexico and northern central American and introduced in Libya for variety purposes, including food fodder, erosion control and afforestation (Alzerbi *et. al.*, 2020). In addition, many reports in the world showed the antifungal capacity of *L. leucocephala* extract against phytopathogenic fungi such as *Asperagillus niger*, *Rhizopus microspores*, *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium solani* and *Alternaria solani* (Abdulrraziq et. Al.,2023) and antibacterial capacity against *Erwinia amylovora*, *Agrobacterium tumefacieus* and *Staphylococcus aureus*. Salem *et. al.* (2011) revealed and identified 44 compounds in *L. leucocephala* by gas chromatography mass spectrometry such as: 2(H)-benzofuranone one-5,6,7,7a- Tetrahydro-4.4.7a – trimethyl (23.1%), pentadecanoic acid- 14- methyl- methyl ester (82%), and 6,10,14- trimethyl-2-pentadeca none acetone (4.2%) were principles chemical constituents. *Anabasis aphylla* is species of flowering plants in the family Chenopodiaceae , native to the region surrounding the Caspian central Asia and Xinjiaug and Western Gansu provinces of china. Previous studies reported that *A. aphylla* have a strong antimicrobial, antibacterial and antifungal biological properties and revealed the presence of alkaloids, flavonoid, saponin, terpenoid, steroid and sterols (Shakeri *et al.*,2011). In the same family *Atriplex halimus* plant commonly known as salt bush and orach and distributed in Asia, Australia, Africa and North American. Previous chemical investigations of *A. halimus* releaved the presence of flavoids, tannins, polyphenols, coumarin …etc (Ounaissia *et. al.*, 2020). Abd-Ellatif *et. al*. (2011) reported the efficiency of methanolic extract of *A.halimus* against *Alternaria alternate*, *Bipolarisoryzae chetomium*, *Rhizopus*, *Fusarium oxysporium*, *Fusarium solani*, *Mucor* and *Pythium ultimum.* In Morocco *A. halimus* aqueous extract at 5% showed the high activity as insecticidal against *Datylopius opuntiae* (Cockerell) under laboratory and greenhouse conditions (Naboulsi *et. al.*, 2022). *Ephedra alta*

(Ephedraceae) perennial herb. The native land for this plant Jordan, Saudi Arabia, Iran, Iraq, Syria, Libya, Mauritania, Morocco and Tunisia (Al-Qarawi *et. al.*, 2014). Several reports have documented the efficiency of E. genus against *F. oxysporium* (Belaidi *et. al*., 2020 and Qarawi *et. al.*, 2014) and *Asperagillus flavus* (Deabes *et. al*., 2020). The plant active materials such as Tannins, saponin, flavoids, cardiae glycosides and alkaloids were recorded by Edrah *et. al.*, 2016. *Arum cyreniacum* belong to Araceae family and native to Europe northern Africa and western central Asia, Mediterranean region has the most species variety. Based on a review of the literature on the chemical consitituents of Arum genus , it was demonstrated the presence of flavoids, alkaloids, terpenes, carbohydrate and sterols (Abdel Karim *et. al*., 2018). Ben Ramadan *et. al.* (2021) documented the antibacterial efficiency of *A. cyreniacum* Against number of medically important pathogenic bacteria while Abdullrazziq *et. al.*, 2023 reported that this plant had antibacterial activity against *Xanthomonas campestris pv. Vesicatoria* which cause tomato spots diseases. *Artemisia annua l.* (Asteraceae) is annual herb native of Asia. Many secondary metabolites such as artemisinin, artemisinic acid and coumarins are characterized from this herb (Ma *et. al*., 2019).The antifungal activity of aqueous extract and essential oil of *A. cyreniacum* herb alba tested on some specific strong storage fungi (*Fusarium sp*. And *Alternaria sp*.) and inhibited fungal growth at 30% concentration, also aqueous extract showed better efficiency than essential oil on *Fusarium* and *Alternari* (Salhi *et. al.*, 2019). The methanol extract from *A. annua* leaves showed strong antifungal activity against *F. oxysporium* and *F. solani* (Ma *et. al.*, 2019). Finally, *Urtica urens* (Urticacea) is widely distributed in Libya.The water extract of *U. uerns* reduced mycelial growth and spore germinatin of *A. alternate, A. solani f. sp. Lycoperisi, Fusarium oxysporium f. sp. Lycoperisi* and *R. solani* (Baka,2014) . Nassar (2016) found that petoleum ether,ethyl acetate and ethanol extract of *U. urens* efficiently controlled a wide world nematodes *Meloidogyne incognita* (root-knot nematodes) and the biological properties related to its richness on various bioactive compounds such as polyphenols, caffeic acid and chlorogenic acid (Mzid *et. al*., 2017).

The gole of this study is to evaluate the aqueous, methanl and hexan extracts from seven Libyan medicinal plants used in traditional medicine against *Asperagillus niger*, *Penicillium itallicum*, and *Rhizopus stolonifer* to their potential application as alternative nontoxic antifungal agents.

Materials and methods:

Plant materials:

Leaves of *Arum cyrenaicum*, *Artemisia anuna*, *Atriplex halimus*, *Ephedra alta*, *Leuceana leucocephala*, *Anabasis aphylla* and *Urtica urens* that tabulated in Table (1) were collected from Al-Jabal El-Akhter region northeast Libya (El-Barasi *et al*. 2003) in spring. They were washed with distilled water to remove all dust and soil particles and allowed to dry 24h at 40-60 C in oven (Nontchit, 2010) grinded and saved for used.

Preparation of plant extracts:

Aqueous extraction : 100 grams of each plant with 1000ml distilled water soaking with shakering for 24h at room temperature. Plant extracts were filtered through two pieces of cheese cloths and clarified by centrifuge at 3000rpm for 10min. Then filtering with filter bacterial what man NO.22mm paper by using vacuum pump (Baka 2014). Methanol and hexanol extraction: 200 ml 0f 99,9% methanol were added on plant materials that

Table 1: list of tested plants and their common names and families.

Scientific name	Common name	Family
Leucaena leucocephala	Jumbie Bean, Jumbay , Lead Tree, Reuse Wattel, White Popinac	Fabaceae
Anabasis aphylla		Chenopodiaceae
Atriplex halimus	salt bush and orach	Chenopodiaceae
Arum cyreniacum	Cyrenaicum Arum Cyrenaicum Calla Lily Cyrenaicum's Arum	Araceae
Artemisia annua	sweet wormwood,[2] sweet annie, sweet sagewort, annual mugwort[3] or annual wormwood,	Asteraceae
Urtica urens	annual nettle, dwarf nettle, small nettle, dog nettle, or burning nettle	Urtica urens (Urticacea)
Ephedra alta	Alenda- White shrubby horsetail.	Ephedraceae

remaining in the filter paper of water extract, shakered for 24h at 35C and centrifuged at 3000rpm for 10min. then dried in rotary evaporator to remove solvent. The extract was resolved with distilled and sterilized water 200 ml and stored at 4C in air tight bottles for subsequent. Likewise, the same steps were applied to the hexane solvent on the remains of the plant material after it was extracted with methanol and stored as mentioned above (Abdullrazziq et. al., 2023).

Source of fungi:

All fungal isolates were previously identified in the Plant Protection Department/ Faculty of Agriculture/ Omar Al--Mukhtar University (table2). These isolates were subculture on Potato Dextrose Agar (PDA) at 25c°. Young pure cultures (7-10 days) of each isolate was used to conduct this study.

the inhibitory effect of plant extracts:

The efficiency of seven medicinal plant extracts with various solvents (water, methanol and hexane) were mixed with sterilized Potato Dextrose Broth medium to prepare 10% concentration of plant extracts in 60 ml and distrebuted into three Erlenmeyer flasks (25 ml). A 5 mm diameter agar disc from growth of each isolate was transferred to each flask and incubated at 20C° for 7 days. Three flasks were prepared for each treatment with three replicates. In addition to three replicate without supplementation of plant extracts saved as control. After incubation the content of each flask was filtered through pre weighted filter paper Whatman NO.1. Mycelial mat with filter paper were dried for 24h at 60C° and reweight. Dry mycelial weight was calculated by subtracting the initial weight of filter paper from final weight of filter paper with the mycelial mat (Kandhare, 2015):

$$\text{Inhibitory effect for each treatment } \% = \frac{of\ control - dry\ weight\ of\ treatment}{dry\ weight\ of\ contro} \times 100$$

Statistical Analysis:

The study experience was designed according to the complete random design (CRD). Statistical analysis was performed using Minitab17 program and ANOVA variance analysis tables. The average were compared using Tokays test at P≤ 0.05 (Abdulrraziq *et al.*, 2023).

From the data studied, you have a factorial experiment on three factors: fungi, plants, solvents... Where is the interaction for the analysis to be complete?

Result and discussion:

The efficiency of seven medicinal plants of *L. leucocephala A. aphylla, A. cyreniacum, A. halimus, A. annua, E. alta* and *Urtica urens* were demonstrated against mycelial growth of *Asperagillus niger*, *Penicillium itallicum*, and *Rhizopus stolonifer* . All tested plant extracts effective in reducing the mycelial growth of all fungi compared with control but to varying degree (Table 2). Several studies showed to the activity medicinal plants in inhibition fungal growth (Baka 2014, Zhang *et. al.*,2023, Naboulsi *et. al.*, 2022). The inhibitory effect of tested plant might be due to phytochemicals present in plants. Abdel rahman *et al.*,2011, Du *et al.*, 2009 and Baka 2014). Several these secondary metabolites act as antimicrobial. Zhang *et al.*, 2023 suggested this result might from loss of rigidity and collapse of fungal mycelial, cross the cell membrane into interior of cell affecting critical intercellular function and impairment of enzymatic processes involved in energy production.

Table 2: The inhibitory rates (%) of aqueous, methane and hexane plant extracts against *Asperagillus niger*, *Penicillium itallicum*, and *Rhizopus stolonifera*.

Plants	*A. niger*			*P. itallicum*			*R. stolonifera*		
	W	M	H	W	M	H	W	M	H
E. alta	66.2abc	59.4 a-d	30.4 o-s	60.2 a-d	56.8 a-g	32.5 k-s	56.1 a-g	53.1 a-j	27.1 p-s
A. cyrenaicum	51.4 b-k	54.4 a-h	31.4 m-s	26.0 q-s	39.8e-q	45.1 d-p	34.7 j-s	45.8 d-p	25.8 q-s
L. leucocephalla	70.7 a	60.4a-d	27.5 p-s	54.7 a-h	50.7 c-l	27.4 q-s	56.4 a-g	54.2 a-h	38.3 g-r
A. aphylla	60.5 a-d	53.7 a-i	31.9 l-s	50.1 c-m	21.4 q-s	21.3 q-s	47.6 c-o	55.6 a-g	29.8 o-s
A. halimus	57.3 a-f	52.7 a-j	28.1 p-s	18.8s	19.5 rs	30.4 o-s	35.0 h-s	59.5 a-d	36.4 h-s
A. annus	49.5 c-n	55.9 a-g	31.3 m-s	30.1 o-s	57.7 a-f	25.2 q-s	39.1 f-q	55.7 a-g	18.6 s
U. urens	69.6 ab	54.4 a-h	29.2 o-s	61.9 a-d	60.2 a-d	30.7 n-s	58.2 a-e	59.9 a-d	25.6 q-s

In our study *Ephedra alta* extract was the most effective against tested fungi (figure1). This result in agreement with that reported in Saudi arabia by Qarawi *et al.*, (2011), who reported that *A. alta* aqueous extract showed highest activity inhabitation of mycelial growth and conidia production as well as Aflatoxin production of *Asperagillus flavus*. On other hand Belaidi *et al.*, 2020 proven the efficiency of the methanol and ethanol extracts of *E. alta* against three *Fusarium F. sp. Albedinis* strains of the causal agent of Bayout disease on date plam.

Likewise *Urtica urens* extract came second to *E. alta* extracts in effectiveness, our result agree with those reported by Baka (2014) who reported that water extract this plant inhibited mycelial growth and spore germination by 10% concentration for *Alternaria alternate f.sp. lycopersici, A. solani, Fusarium oxysporium f.sp. lycopersici* and *Rhizoctonia solani*

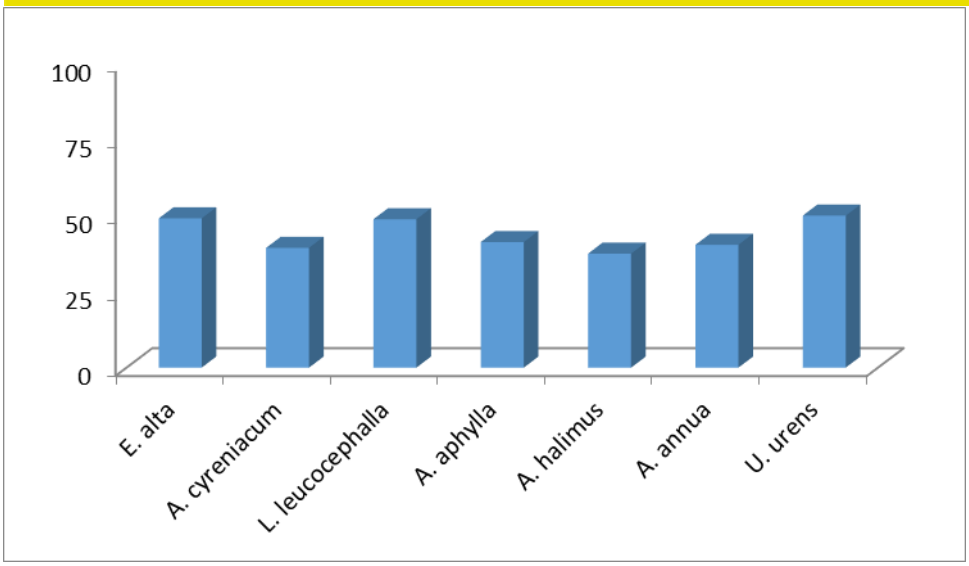


Figure 1: Inhibition rates (I%) of mycelial growth of tested fungi by water, methanolic and hexanol extract effect of different plants.

The inhibitory effect of tested plant might be due to phytochemicals present in plants abdel rahman *et al.*, 2011 , Du *et al*., 2009 and Baka 2014). Several these secondary metabolites act as antimicrobial. Zhang *et al*., 2023 suggested this result might from loss of rigidity and collapse of fungal mycelial, cross the cell membrane into interior of cell affecting critical intercellular function and impairment of enzymatic processes involved in energy production. In our study *Ephedra alta* extract was the most effective against tested fungi. This result in agreement with that reported in Saudi arabia by Qarawi *et al.*, (2011), who reported that *A. alta* aqueous extract showed highest activity inhabitation of mycelial growth and conidia production as well as Aflatoxin production of *Asperagillus flavus*. On other hand Belaidi *et al.*, 2020 proven the efficiency of the methanol and ethanol extracts of *E. alta* against three *Fusarium F. sp. Albedinis* strains of the causal agent of Bayout disease on date plam. *Urtica urens* extract came second to *E. alta* extracts in effectiveness, our result agree with those reported by Baka (2014) who reported that water extract this plant inhibited mycelial growth and spore germination by 10% concentration for *Alternaria alternate f.sp. lycopersici, A. solani, Fusarium oxysporium f.sp. lycopersici* and *Rhizoctonia solani. Anabasis aphylla* extract was the less effective against tested fungi. Although Shaker *et al.*, (2012) revealed the presence of alkaloids, flavonoids,saponin,terpenoid,steroid and sterol in the aerial part of that plant. Furthermore, the efficiency of n- butanual A. aphylla extract against *A. niger* at 25-100% mg/ml. The remained of plant extracts exhibited moderate antifungal activities compared to control (figure 1), and some even enhanced the mycelial growth of tested fungi as reported by Meziane et al., (2007). *Asperagillus niger*, and *Rhizopus stolonifer* fungi were the most affected fungi for all tested plant extracts while *Penicillium italicum* fungus was the least affected (figure 3) for all tested extracts (Pillai *et al.*, 2020).

On the other hand, the result indicated that the aqueous and methanol extracts gave more efficiency than hexane extracts (Figure2). The solvent polarity has important effect on release of most secondary metabolites (Mazandarani *et al.*, 2012 and Abubkar and Haque 2019). In previous studies, the hexane extract of the aerial parts of *Atriplex halimus* plant

showed less activity against 15 gram positive and negative pathogenic bacteria compared to methanol extract (Ramadan *et al.*, 2011), while the ethanol extract of the aerial parts of Tunisia of *U. urens* showed the highest value of total polyphenol, flavonoids and tannin content compared to the water extract (Mzid *et al.*, 2017).

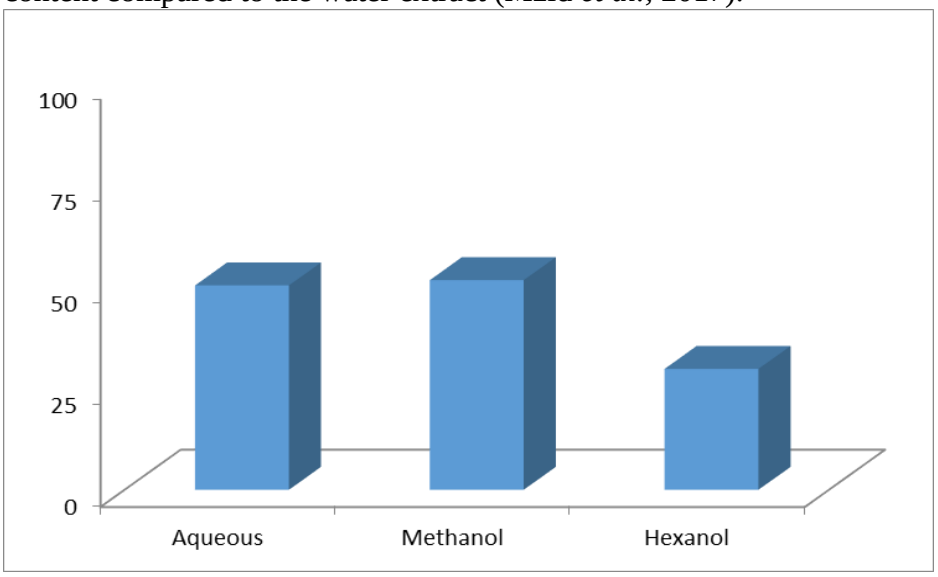


Figure2: effect of of solvent type on inhibitory effect of plants.

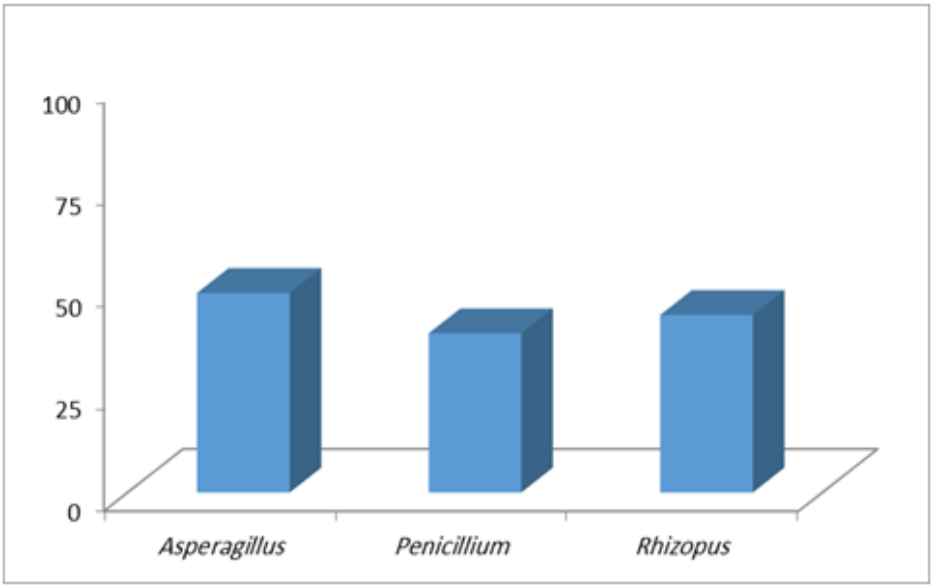


Figure3:Sensitivity of tested fungi for treatments.

Also the species of plant can effect on that phytochemicals, Deabes *et al.*, 2022 recorded that *E. sinica* extract has significant inhibition (73.3-94.6%) for *Asperagillus flavus*, while Khan *et al.*, 2017 reported that *E. gerardiana* extract was non active against *A. niger* and *A. flavus*. In addition to the effect of soil and the climatic conditions as well the solvent and method of extraction on phytochemicals extraction.

Conclusion:

In conclusion, all tested plant extracts effective in reducing the mycelial growth of all fungi compared with control but to varying degree The inhibitory effect of the tested plant may be due to phytochemicals present in plants and recorded in previous studies.

On the other hand, the results indicated that aqueous and methanolic extracts gave greater efficiency than hexanic extracts.

In our study it was extracted Ephedra alta is most effective against the fungi tested. Anabasis aphylla extract was the least effective against the fungi tested.

Asperagillus niger, Botrytis cinerea and Rhizopus stoloniferare the most affected fungi of all plant extracts tested especially Leucaena leucocephala.

Recommendations:

1. Identification and purification of active substances in Alanda, Lucinia and Fire extract responsible for antifungal activity.
2. Urging researchers to conduct future studies of plant extracts tested as an alternative and safe method in control of plant diseases (bacteria - fungi - nematodes).
3. Conducting studies to confirm the safety of consumers from the effect of these extracts if agricultural products.

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