Multi-Knowledge Electronic Comprehensive Journal For Education And Science Publications(MECSJ)
Issues 80 (2024)
ISSN: 2616-9185

Effect of dried Henna leaf extract on the growth of bread mold fungus

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Abstract

The study conducted in Bisha province south Saudi Arabia, about the effect of dried Henna leaf extract on the growth of bread mold fungus. The main objective of the study to identify the effect of the aqueous extract of Henna leaves on inhibiting the growth of mold fungi, the important of the research focus on the using of henna leaves in human life, socially and ornamental plant constitute the volume of cultivate plant that are important in agricultural activities in various parts of the world, because of their economic aesthetic value. Also the study answers the questions related to the topics for example Henna extract destroyed the fungi. The Henna leaf powder was purchased from the local market of Bisha city. The methodology used laboratory experiments, add to description approach, the data analysis use percentage methods and tools (Al-Janabi 1998). We prepared five different concentrations for this extract (5, 8, 11, 17 and 19). The study detected antimicrobial activity by using well diffusion in agar technique against all the Fungi molds (Rhizopus). The study showed highest antibacterial activity of aqueous extract of henna leaves which attained the highest present of inhibition (32 mm) against genus rhizopus fungi when using (19%) concentration, (21mm) when using (17%), (9mm) when using (11%), (7mm) when using (8%), While attained lowest percent of inhibition (4mm) on concentration (5%.). The main recommendation, avoid eating or pollution with Henna extracts especially children, due to higher effective materials produce toxins and essential oils, and also use in inhabited the growth of molds fungi.

Keywords: Henna, extract, Rhizopus, molds fungi.

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الملخص

أجريت هذه الدراسة في محافظة بيشة جنوب المملكة العربية السعودية حول تأثير مستخلص أوراق الحناء المجففة على نمو فطر عفن الخبز، وكان الهدف الرئيسي من الدراسة هو التعرف على تأثير المستخلص المائي لأوراق الحناء في تثبيط نمو فطر عفن الخبز، وتركز أهمية البحث على استخدام أوراق الحناء في حياة الإنسان، اجتماعياً وكنباتات زينة التي تشكل حجم النباتات المزروعة والتي لها أهمية في الأنشطة الزراعية في مختلف أنحاء العالم، لما لها من قيمة جمالية واقتصادية.

كما أجابت الدراسة على الأسئلة المتعلقة بالمواضيع مثلا مستخلص الحناء مثبط لنمو الفطريات. تم شراء مسحوق أوراق الحناء من السوق المحلي لمدينة بيشة، المنهج المستخدم هو التجارب المعملية، إضافة إلى المنهج الوصفي، وتحليل البيانات باستخدام أساليب وأدوات النسب المئوية (الجنابي 1998). تم تحضير خمسة تركيزات مختلفة لهذا المستخلص (5، 8، 11، 17 و19). وقد كشفت الدراسة عن النشاط المضاد للميكروبات باستخدام تقنية الانتشار في الاجار. وقد أظهرت الدراسة أعلى نشاط مضاد للفطريات عند استخدام المستخلص المائي لأوراق الحناء والذي حقق أعلى نسبة تثبيط (32 ملم) ضد فطريات جنس الريزوبس عند استخدام تركيز (19%)، (21مم) عند استخدام تركيز (17%)، (9مم) عند استخدام تركيز (11%)، (7مم) عند استخدام تركيز (8%)، بينما تم تحقيق أقل نسبة تثبيط (4مم) عند تركيز (5%). والتوصية الرئيسية تجنب تناول أو التلوث بمستخلصات الحناء خاصة الأطفال، بسبب المواد الفعالة العالية والسموم التي تنتجها والزيوت الأساسية، وتستخدم أيضا في تثبيط نمو الفطريات المأهولة.

الكلمات المفتاحية: الحناء، المستخلص، فطريات العفن، الريزوبس.

Introduction:

The study attended to the important use of Henna powder in human life and the materials composes it. On the other hand, its attempt to observes the Henna extracts inhabitants the mold fungi, add to the rare of this type of studies. The study of (Demet.Y. 2017) its modern and similar study about the effect of Henna extract at medical diseases fungi, study of the

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effects of henna extracts (Lawsonia inermis) on Malassezia species, resulted that aqueous extract is more effective on Malassezia than methanolic and chloroformic extracts. (Fariba Berenji and others. 2010).

Although (Mohamed. Abbas. 2022) the results indicated that Henna extracts inhabitant the growth of fungi in varieties levels, however, in other study which obtained results revealed antifungal activity of Henna leaves extracts which support the traditional use of Henna in therapy of fungal infections Elham. S, Albasheir.M.2014).

Lawsonia inermis, also known as Henna, the henna tree, the mignonette tree, and the Egyptian privet, is a flowering plant and one of the only two species of the genus Lawsonia, with the other being Lawsonia odorata. The species is named after the Scottish physician Isaac Lawson, a good friend of Linnaeus.

Kingdom: Plantae
Order: Myrtales
Family: Lythraceae
Scientific name: Lawsonia inermis

Botanical description: Lawsonia inermis is a much-branched glabrous shrub or small tree 2-6 m in height, which may be spiny. Bark greyish-brown, unarmed when young, older plants with spine-tipped branch lets. Young branches quadrangular, green but turn red with age. (Bele A.A., Jandhav 2010).

Leaves opposite, entire, sub sessile, elliptic to broadly lanceolate, 1.5-5 x 0.5-2 cm, glabrous, acuminate; veins on the upper surface depressed.

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Flowers small, white, numerous; in large pyramidal terminal cymes, fragrant, 1 cm across, 4 petals crumpled in the bud. Calyx with 2-mm tube and 3-mm spread lobes; petals orbicular to obovate, white or red; stamens 8, inserted in pairs on the rim of the calyx tube; ovary 4 celled, style up to 5 mm long, erect. Fruits small, brown, globose capsules 4-8 mm in diameter, many-seeded, opening irregularly, split into 4 sections, with a persistent style. Seeds 3 mm across, angular, with thick seed coat. The specific epithet means unarmed or without spines.

Ecology: inermis is widely distributed throughout the Sahel and into Central Africa; it also occurs in the Middle East. It grows mainly along watercourses and in semi-arid regions and is adapted to a wide range of conditions. It can withstand low air humidity and drought. Henna requires high temperatures for germination, growth and development.

BIOPHYSICAL LIMITS: Mean annual temperature: 19-27 deg. C, mean annual rainfall: 200-4200 mm Soil type: Prefers sandy soils but can tolerate clays and poor, stony, sandy soils; optimum soil pH is 4.3-8.

Species Distribution

The map under shows countries where the species has been planted. It does neither suggest that the species can be planted in every ecological zone within that country, nor that the species cannot be planted in other countries than those depicted. Since some tree species are invasive, you need to follow biosafety procedures that apply to your planting site. Exotic range Native range Algeria, Cyprus, Egypt, Eritrea, Ethiopia, Indonesia, Iran, Iraq, Jordan, Kenya, Kuwait, Lebanon, Libyan Arab Jamahiriya, Malaysia, Morocco, Oman, Philippines, Qatar, Saudi Arabia,

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Syrian Arab Republic, Tanzania, Tunisia, Turkey, Western Sahara, Yemen, Republic of Australia, Benin, Burkina Faso, Cameroon, Central African Republic, Chad, China, Congo, Cote d'Ivoire, Democratic Republic of Congo, Gabon, Gambia, Ghana, Guinea, India, Liberia, Mali, Mauritania, Niger, Nigeria, Pakistan, Senegal, Sierra Leone, Spain, Sudan, Togo, Zanzibar(Demet YİĞİT -2017)

Figure.1) The map above shows countries where the species has been planted.

It does neither suggest that the species can be planted in every ecological zone within that country, nor that the species cannot be planted in other countries than those depicted. Since some tree species are invasive, you need to follow biosafety procedures that apply to your planting site.

Products: Fodder - Leaves of interims are browsed by livestock.

Fuel: Henna is a suitable source of firewood.

Timber: The wood of Henna is fine grained, hard, and is used to make tent pegs and tool handles in India. Fibber: In Turkana, Kenya, the stems are used for making fishing baskets. Tannin or dyestuff: An orange-red dye is made by crushing the leaves and younger shoots to a grey-green powder. The powder is soaked in a mixture of strong tea and lemon juice

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and is used in Sudan, Middle Eastern countries and many Muslim communities in Africa for decorating hands, nails and feet with patterns. Henna is also used as a hair dye and conditioner as well as a coloring agent for leather and cloth. It may also be used to stain wood. Medicine: Roots are regarded as a potent medicine for gonorrhea and to enhance fertility in women; a decoction of them is considered to be diuretic or for treating blenorrhoea and pectoral for bronchitis. A reported constituent of the leaves is an ox naphthoquinone called laws one, which has antibiotic properties. Flower oil contains alpha- and beta ionone, the latter being the main component. Leaf and flower infusions are applied externally for ulcers and rheumatism or are taken orally for tetanus, epilepsy and stomach pains; leaves are used in treatment of leprosy, jaundice and scurvy. Astringent roots are ground and rubbed on the heads of children to treat boils and eye diseases.

Other products: The fragrant flowers are used as perfume, while small twigs are used as toothbrushes in Indonesia.

Definition of mold fungi

Molds are microscopic fungi that live on plant or animal matter. They can be found indoors and outdoors and are part of our natural environment. They play an important role in the environment by breaking down and digesting organic material. Also called fungi or mildew, molds are neither plants nor animals; they are part of the kingdom Fungi. (Forbes, B.A. (1998).

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Molds can multiply by producing microscopic spores similar to the seeds produced by plants. Many spores are so small they easily float through the air and can be carried for great distances by even the gentlest breezes.

Molds come in many colors including white. "Black mold" is not a species or specific kind of mold, and neither is "toxic mold." Sometimes the news media use the terms "*toxic mold*" and "*black mold*" to refer to molds that may produce mycotoxins or for a specific mold, *Stachybotrys chartarum*. Molds that produce mycotoxins are often referred to as toxigenic fungi. (Demet YİĞİT (2017).

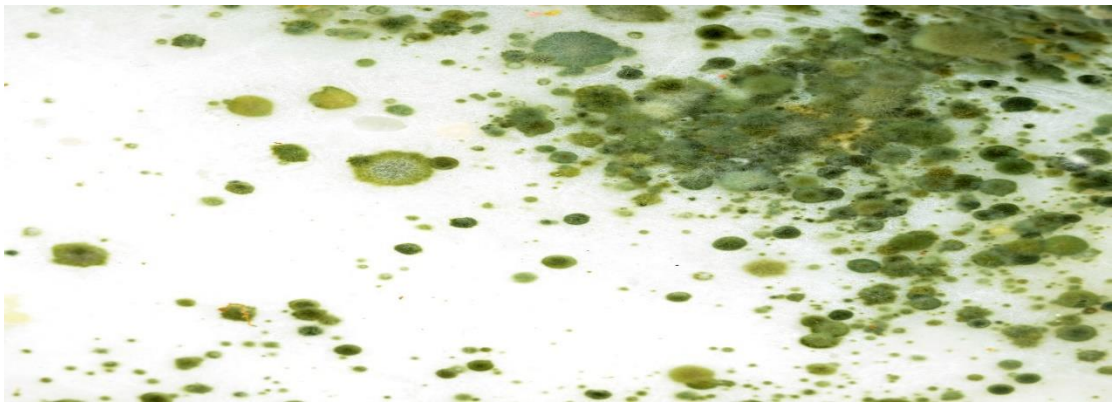

Figure .2. Mold fungi structure

Mold is not usually a problem indoors - unless mold spores land on a wet or damp spot and begin growing. As molds grow they digest whatever they are growing on. Unchecked mold growth can damage buildings and furnishings; molds can rot wood, damage drywall, and eventually cause structural damage to buildings. Mold can cause cosmetic damage, such as stains, to furnishings. The potential human health effects of mold are also a concern. It is important, therefore, to prevent mold from growing indoors.

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Definition of fungi included in the study:

Rhizopus:

Cosmopolitan genus of some 10 species of filamentous fungi in the family Rhizopodaceae (formerly Mucoraceae), in the order Mucorales. Several species, including *Rhizopus stolonifer* (the common bread mold), have industrial importance, and a number are responsible for diseases in plants and animals. The majority of *Rhizopus* species are saprobic (decomposers) and feed on a variety of dead organic matter, though some species are parasitic or pathogenic. *Rhizopus* fungi are characterized by a body of branching mycelia composed of three types of hyphae.

Figure. 3. bread mold *Rhizopus stolonifer*, a species of bread mold, produces sporangia that bear sporangiospores (asexual spores).

Stolons, rhizoids, and usually unbranching sporangiophores. The black sporangia at the tips of the sporangiophores are rounded and produce numerous non-motile multinucleate spores for asexual reproduction. *Rhizopus* can reproduce sexually when two compatible and physiologically distinct mycelia are present. The rapidly growing colonies fade from white to dark as they produce spores and are similar to cotton candy (also called candy floss or fairy floss) in texture, commonly

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used in industrial processes. R. arrhizus (R. oryzae) is useful for the production of lactic acid and cortisone, for alcoholic fermentation, and

for the bio sorption (passive adsorption of chemical contaminants by an organism) of heavy metals. *R. stolonifer* is used to produce fumaric acid, lactic acid, and cortisone, and *R. delemar* produces fumaric acid and biotin. In Asia several species are important in some foods, such as tempeh, and in many traditional alcoholic beverages.

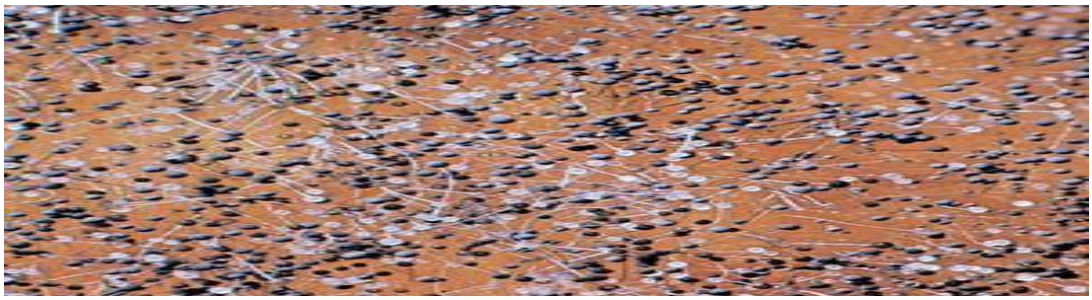

Figure. 4. peach mold Magnified view of peach mold (*Rhizopus*).

Medicinal Plants and their active ingredients

The active ingredients of medicinal plants consist of well-known chemical groups such as alkaloids and glycosides, or they can be complex mixtures of derived organic compounds, such as volatile oils, resins, alkaloids, and resins. (Al-Qureshi-Manar -2011).

Research problem:

The research problem is concentrated in the following questions:

1- Does the aqueous extract of Henna leaves inhibit the growth of false mold fungi?

2- What are the active substances in the leaves of the Henna plant?

Objectives of the study:

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1 - Identify the effect of the aqueous extract of Henna leaves on inhibiting the growth of mold fungi.

2 - Knowing the active substances in Henna extract leaves and how sensitive to mold fungi.

Materials and methods:

1 - Collection of plant samples

The Henna leaf powder was purchased from the local market of Bisha city and taken to the plant laboratory at the College of Science, Bisha University, for the purpose of preparing the Henna leaf extract. The data analysis use percentage.

2 - Fungal isolation

Bread mold fungus was isolated from food and bread residues after about 72 hours, where the fungus grew on it in addition to observing fungal spores on it. They were surface sterilized by washing with either ethanol - water (80:20 v/v) or sodium hypochlorite - water (3.5% w/v). Air-dried and plated on potato dextrose agar (PDA, Oxoid, Basingstoke, UK). Two plates per sample were prepared. After incubation for 2- 5 days at 25°C, the primary fungal colony was transferred to a PDA plate, purified as required and stored on PDA slants at 4°C.

3 - Preparation the aqueous of extract

The method was followed in preparing the aqueous extracts by mixing 20 g of the previously prepared dry Henna powder with 100 ml of distilled water in a 500 ml volumetric flask. Then the suspension was left

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in a water bath and shaken at 40 °C for 24 hours. Then the suspension was filtered using several layers of medical gauze and sterilized through a 0.22 μmmilipore filter. Then the clear liquid was stored in tightly sealed containers in the refrigerator at 4 °C until use.

4 - Preparing the aqueous extract concentration:

Testing the effect of plants extracts on henna leaves on the growth of mold fungi the method of (Al-Janabi 1998) was followed by mixing the aqueous extracts of each liquid separately with the PDA medium after it was sterilized and cooled to 50°C and at concentrations of (5, 8, 11, 17 and 19) ml of the food extract /100 ml respectively. At a rate of three repetitions for each concentration. After the food medium solidified, a disk with a diameter of 6 mm was placed for 10 days in a hole of the same diameter in the center of the dish for 7 days on a fungal colony growing on the PDA medium containing one of the aforementioned concentrations, the result is read by measuring the diameters of the inhibition zone, the lower concentration did not appear was considered the minimum inhibitory concentration.

Results and Discussion

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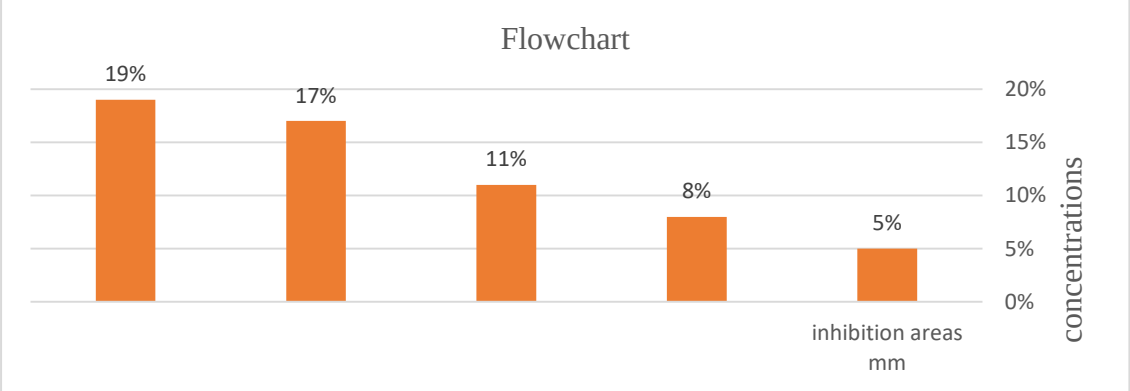
The results of the current study indicate that the aqueous extract of Henna leaves possesses high antibacterial activity under study. This killer effect is caused by the active substances present in the plant and spread in the medium, which helped kill mold fungi and inhibit the growth of living cells.

The study demonstrated that the aqueous extract of henna leaves was given the largest inhibition regions if they reached (32 mm) at concentration (19 %) towards genes riphzopus, and the lowest inhibition levels when reached (4 mm) when using the concentration (5%). As shown in Table No. (1) below.

Table No. (1) Antimicrobial activity of Henna extractor leaves:

inhibition areas mm					**Types of Fungi**
5%	8%	11%	17%	19%	concentrations
4mm	7mm	9mm	21mm	32mm	Riphzopus *stolonifer*

Figure No. (5) Antimicrobial activity of Henna extractor leaves



So far, no study has been undertaken to investigate the antifungal properties of Henna so this study aims to evaluate dyeing characteristics

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and antifungal efficacy of Henna extract processed with against bread molds. Also, the study its similar than the study of (Demet YiGiT- 2017) about henna paste showed the highest antifungal activity against 68 (35.4%) clinical *Candida* isolates (≥20 mm inhibition zone). Other study resulted that (Fariba Berenji & others (2010) the Henna has antifungal activity against Malassezia. In addition, aqueous extract is more effective on Malassezia than methanolic and chloroformic extracts.

Conclusion:

When the concentration of the extract increases, the degree of inhibition increases and vice versa. We conclude that the henna leaf powder extract has an inhibitory effect on the growth of bread mold fungi.

Recommendation:

More studies about the effects of Henna extract leaves on other fungi, avoid eating or pollution with Henna extracts especially children, due to higher effective materials produced toxins and essential oils, and also use in inhabited the molds fungi.

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