Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2023; 4(1): 11-15

www.microbiojournal.com
Received: 06-10-2022
Accepted: 13-11-2022

Noor Mohammed Rafie Abdul-Jabbar Shanshola
Department of Biology, College of Education for Girls, University of Mosul, Mosul-Iraq

Dr. Abdul Karim Sulaiman Hassan Al-Nuaimi
Department of Biology, College of Education for Girls, University of Mosul, Mosul-Iraq

Correspondence
Noor Mohammed Rafie Abdul-Jabbar Shanshola
Department of Biology, College of Education for Girls, University of Mosul, Mosul-Iraq

Inhibition of the growth of the pathogenic fungus *Trichophyton rubrum* by *M. azedarach* leaves

Noor Mohammed Rafie Abdul-Jabbar Shanshola and Dr. Abdul Karim Sulaiman Hassan Al-Nuaimi

DOI: https://doi.org/10.22271/micro.2023.v4.i1a.58

Abstract
The effect of the alcoholic and aqueous extract of the leaves of *M.azedarach* plant against the growth of the pathogenic fungus *T. rubrum* was studied, and the results showed that the alcoholic extract of the leaves of the *M.azedarach* plant was more efficient than the aqueous extract in inhibiting the growth of the pathogenic fungus *T. rubrum*, as it completely inhibited the growth of 0.00 cm at concentrations (15-20-25) mg/ml. As for the concentrations (5-10) mg/ml, the average diameter of the colony after an incubation period of 14 days was (4.25-1.25) cm, respectively, while the aqueous extract of the leaves of *M.azedarach* plant had an effect on the growth of the pathogenic fungus *T. rubrum*, as the average colony diameter was (6.25-2.25) cm for concentrations (5-10) mg/ml, respectively, after a 14-day incubation period.

Keywords: Pathogenic fungus, *Trichophyton rubrum*, *M. azedarach leaves*

Introduction
Dermatology diseases are one of the common health problems that affect 20-25% of the world's population (Chowdhary *et al*., 2022) [5], it is a group of fungi that have the ability to attack keratinized tissues of humans and animals such as hair, skin and nails (Faway *et al*., 2021) [8], causing superficial fungal infections of the skin known as Dermatophytosis (Al harbi *et al*., 2022) [4], and this group includes three genera: Epidermphytin, Trichophyton, Microsporum (Janardhan, 2017) [13].

Because of the importance of dermatophytic fungi and the difficulty of eradicating them, it became necessary to search for therapeutic alternatives, therefore, medicinal plants were used as therapeutic alternatives, which gained wide importance in the treatment of many diseases. Plants are considered a repository for many effective compounds with a medicinal effect, including alkaloids, phenols, flavonoids, proteins, terpenes, saponins, volatile oils and others (Qutub, 1989) [2]. The Melai azedarach tree is considered one of the most famous economic trees, which are found in tropical and subtropical regions such as China, Iraq and some other countries (Feng *et al*., 2022) [9]. The Melai azedarach tree is grown as a shade and ornamental tree, in addition to its use in folk medicine. It is used as an antioxidant, anti-inflammatory, analgesic, insecticide, rodenticide, diuretic, antidiarrheal, hypotensive and antirheumatic. (Sharma and Paul, 2013) [20].

Therefore, the current study aimed to
Inhibition of the growth of the pathogenic fungus *T. rubrum* by alcoholic and aqueous extract of *M.azedarach* leaves.

Materials and Methods
Clinical sample collection
(53) clinical samples were collected for the period between 1/11/2021 and 1/2/2022, from patients who attended the consulting dermatology clinic in Al-Salam Teaching Hospital and whose infection was diagnosed by the consultant doctors, as the samples were collected after sterilizing the area with 70% medical alcohol by a sterile medical scalpel, by scratching the affected part to obtain scales, and the samples were preserved in sterile dishes and brought to the fungus laboratory in the College of Education for Girls/University of Mosul for

examination and cultivation.

Collecting leaf samples of the *M.azedarach* plant

The leaves of the *M.azedarach* plant were collected from trees in home gardens during October 2021 AD in Al-Mithaq area of the city of Mosul, and after confirming their diagnosis, they were dried well and then ground to obtain a fine powder and kept in dark and clean glass containers in the refrigerator at 4 °C until use in preparing the extracts later (Rios *et al*., 1987) [18].

Direct microscopy and culture of samples

A small amount of skin scraping was taken with a sterile needle and placed on a clean glass slide and a drop of 10% KOH solution was added to it, then the cover of the slide was placed on it and it was passed over the flame three times and left for 10 minutes.

Then, all the glass slides were examined under a microscope using powers of 10X and 40X to note the presence of branched filamentous structures and articular spores, and then they were planted on Petri dishes containing SDA medium, and the dishes were incubated at a temperature of 27± 2°C for 7-14 days (Kwon-chung and Bennett, 1992) [15].

Identification of isolated fungi

The dishes were examined by taking a part of the fungal colony using a sterile needle and placing it in a drop of methyl blue dye on the glass slide, then placing the slide cover on it and examining it microscopically using the following taxonomic references (Forbes *et al*., 2002; Jorgensen *et al*., 2005; Eliss *et al*., 2007) [10, 14]

Preparation of alcoholic and aqueous extract of *M.azedarach* leaves

50 gm of *M.azedarach* leaf powder were taken and placed in a beaker, 400 ml of petroleum ether solvent was added to it and placed on a Magnetic Stirrer for 72 hours, then the mixture was filtered and the precipitate was taken and placed in a beaker and 400 ml of ethyl alcohol, with a concentration of 70%, was added to it and left for 72 hours, after which the solution was filtered and kept in the refrigerator (Le^{e}- Grand, 1988) [16], after which the extracts were concentrated with a (RVE) device at 40 °C, then the precipitate was taken again and soaked with (400) milliliters of distilled water with the temperature turned on (40-60) °C to obtain the hot water extract, the extracts were kept in the refrigerator until use (Harborne, 1984) [12].

Sterilization of extracts

The alcoholic and aqueous extracts were sterilized according to the concentrations used on the skin in a water bath at a temperature of 50°C for 15 minutes (Al-Noman, 1998) [3].

Preparation of concentrations and testing of extracts

2 g of alcoholic and aqueous extracts of *M.azedarach* leaves were dissolved in 10 ml of DMSO as a standard concentration for the preparation of dilutions (0.5-1.0-1.5-2.0-2.5) ml and each was added to Petri dishes containing SDA medium before solidification with shaking, with volumes (17.5-18.0-18.5-19.0-19.5) mg/ml as well as a control plate that does not contain additives (Al-Khafaji, 2000) [1]. After hardening of the medium, a tablet was taken from the edge of a fungal colony of *T.rubrum* at the age of 14 days and placed in the center of the plate, the dishes were incubated at a temperature of 27± 2°C for a period of 14 days with recording the inhibitory activity of the extracts by calculating the average of each two orthogonal diameters every 3-6-9-12-14 days.

Statistical analysis

The statistical program SPSS was used to analyze the data of the study, as the Duncan's Multiple Range test (Duncun, 1955) [21] was used to determine the least significant difference at the probability level of 0.05.

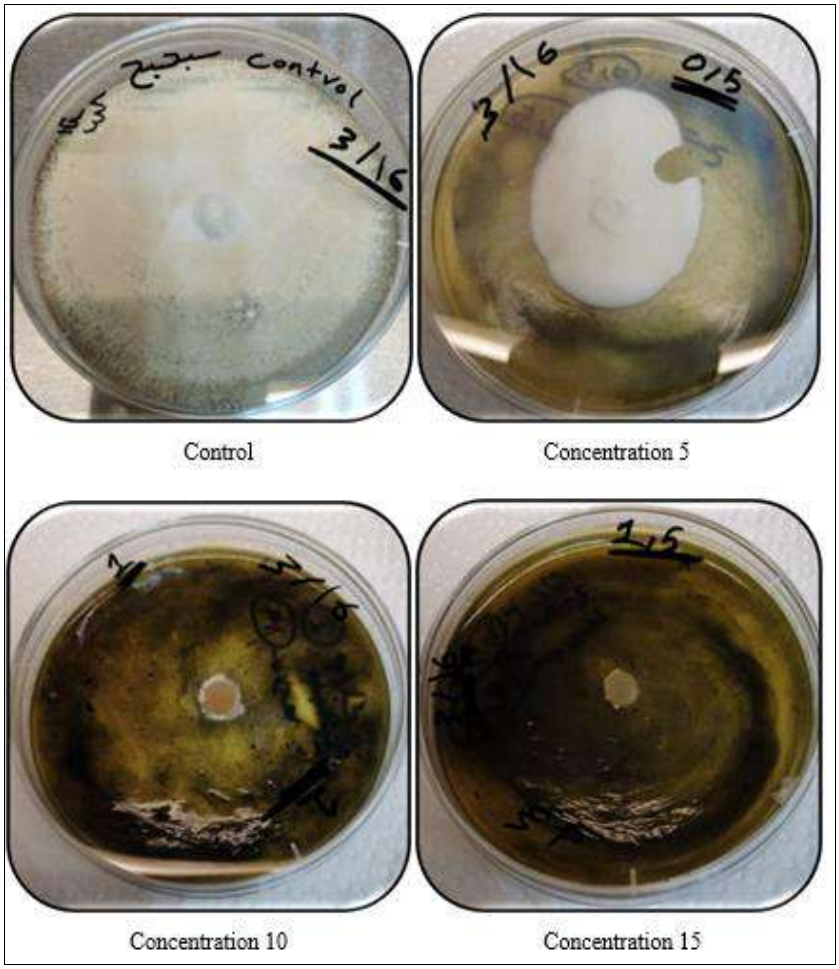
Results and discussion

The study shows the effect of different concentrations (5-10-15-20-25) mg/ml of alcoholic and aqueous extracts of the leaves of the *M. azedarach* plant against the growth of the pathogenic fungus *T. rubrum* on SDA medium. The alcoholic extract of the leaves of the *M. azedarach* plant is more effective than the aqueous extract in inhibiting the growth of the pathogenic fungus *T. rubrum* completely inhibited growth at concentrations (15-20-25) mg/ml and the fungus did not give any growth (0.00) cm compared to the control treatment, while the concentrations (5-10) mg/ml gave inhibition in the growth of the pathogenic fungus *T. rubrum*, as the average diameter of the colony after an incubation period of 14 days was (4.25-1.25) cm, respectively (Table 1).

These results are consistent with (Mindali *et al*., 2009), which showed that the alcoholic extracts of *M.azedarach* plant were highly effective in inhibiting the growth of the fungus Aspergillius SP, Rhizopus SP.

This may be due to the fact that the leaves contain toxic compounds that have an effect on the growth of pathogenic fungi (Goktaset *et al*., 2022) [11], as well as consistent with the findings of (Shanthi *et al*., 2022) [19], which demonstrated the effect of the alcoholic extract of the leaves of *M.azedarach* plant against some species of bacteria that cause skin infections.

As for the aqueous extract of the leaves of *M.azedarach* plant, it completely inhibited the growth of the pathogenic fungus (0.00) cm at concentrations (15-20-25) mg/ml after 14 days. While the pathogenic fungus *T. rubrum* showed growth at two concentrations (5-10) mg/ml was (6.25-2.25) cm, respectively, compared with the control treatment, and these results are consistent with what was reached by (EL-shaer, 1998), which indicated that the effective effect of aqueous extracts of the leaves of *M.azedarach* plant against the growth of a group of fungi that cause rotting cowpea and pea roots in Egypt, and this was attributed to the increase in the proportion of active compounds such as different phenols in the leaves of *M.azedarach* plant.



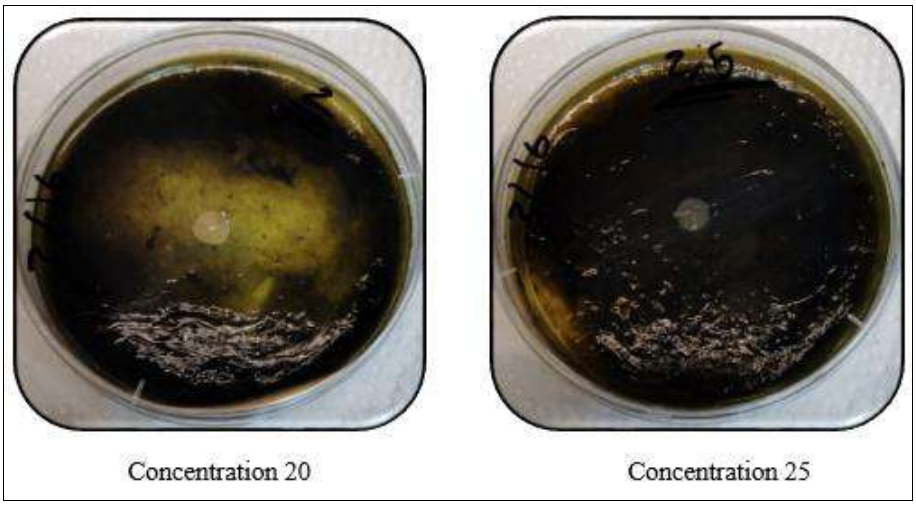


Fig 1: Effect of different concentrations of the alcoholic extract of the leaves of *M. azedarach* plant with different incubation periods on the growth of the pathogenic fungus *T. rurbrum* in the high concentration method.

Table 1: Effect of different concentrations of alcoholic extract of the leaves of *M. azedarach* plant with different incubation periods on the growth of the pathogenic fungus *T. rubrum* in the high concentration method

Incubation period (day) Concentration (mg/ml)		3	6	9	12	14
The effect of the extract on the growth of the pathogenic fungus *T. rubrum* (cm)	**Control**	3.50 g ±0.09129	4.61 i ±0.04595	5.75 j ±0.09129	8.00 k ±0.00000	8.50 l ± 0.00000
	5	1.25 d ± 0.09129	2.50 e ± 0.09129	3.25 f ± 0.09129	3.50 g ± 0.00000	4.25 h ± 0.09129
	10	0.00 a ± 0.00000	0.50 b ± 0.00000	0.91 c ± 0.13944	1.25 d ± 0.09129	1.25 d ± 0.09129
	15	0.00 a± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ±0.00000
	20	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000
	25	0.00 A± 0.00000	0.00 a± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000

- The similar letters between the groups indicate that there are no significant differences between the groups at the level of significance P ≤ 0.05.
- The different letters between the groups indicate that there are significant differences between the groups at the level of significance P ≤ 0.05.

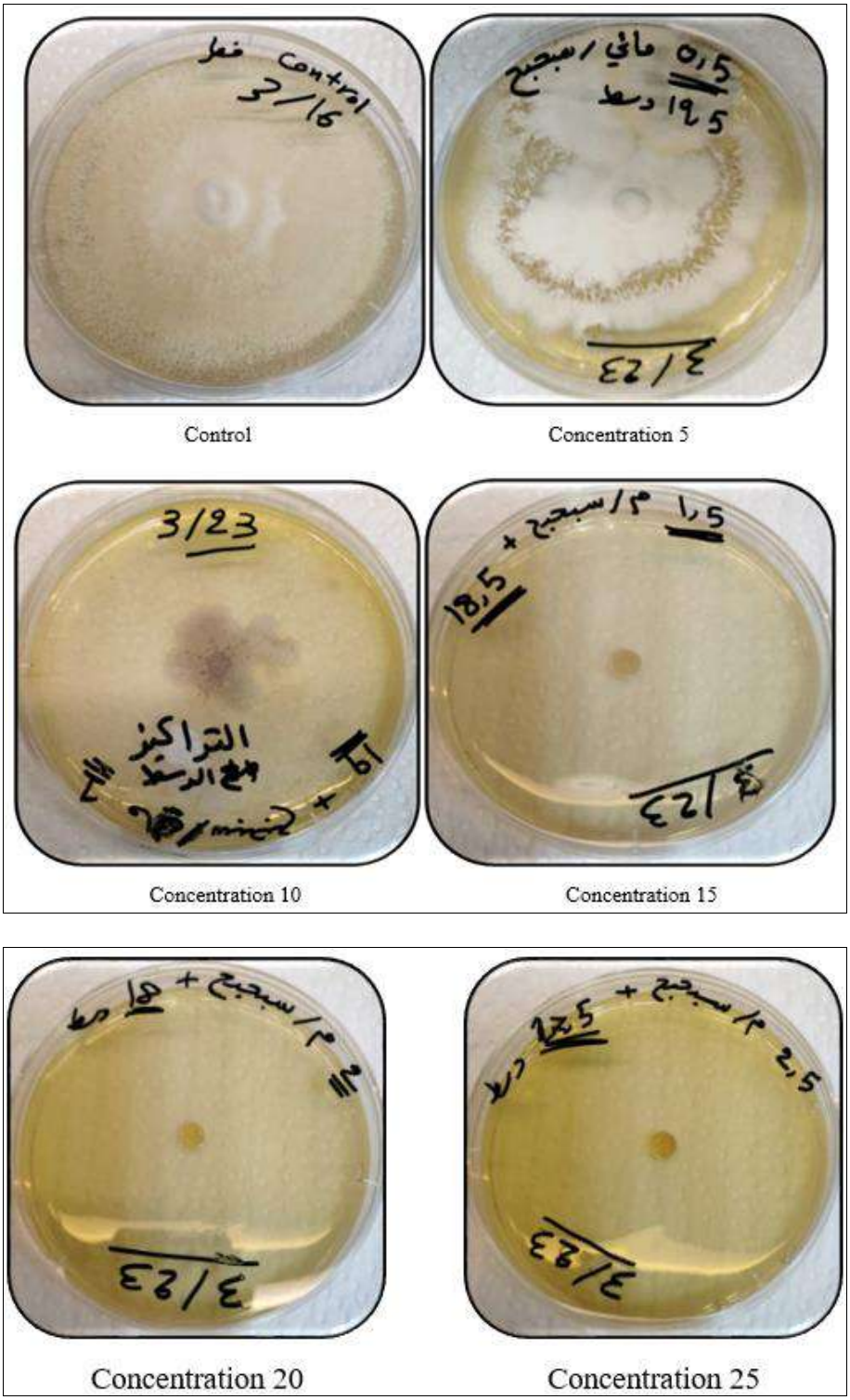


Fig 2: Effect of different concentrations of the aqueous extract of the leaves of *M. azedarach* plant with different incubation periods on the growth of the pathogenic fungus *T. rubrum* in the high concentration method

Table 2: Effect of different concentrations of the aqueous extract of the leaves of *M. azedarach* plant with different incubation periods on the growth of the pathogenic fungus *T. rubrum* in the high concentration method

Incubation period (day) Concentration (mg/ml)		*3*	*6*	*9*	*12*	*14*
The effect of the extract on the growth of the pathogenic fungus *T. rubrum* (cm)	**Control**	2.35 f ± .04830	4.75 I ± 0.09129	5.50 j ± 0.18257	6.75 L ± 0.01929	8.25 m ± 0.09129
	5	1.75 d ± .09129	2.25 ef ± .09129	3.25 g ± 0.09129	4.25 h ± 0.00000	6.25 k ± 0.0000
	10	0.00 a ± 0.00000	0.50 b ± 0.18257	1.50 c ± 0.09129	2.08 e ± 0.05270	2.25 ef ± 0.00000
	15	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000
	20	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000
	25	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000	0.00 a ± 0.00000

The similar letters between the groups indicate that there are no significant differences between the groups at the level of significance $p \leq 0.05$.

The different letters between the groups indicate that there are significant differences between the groups at the level of significance $p \leq 0.05$.

Acknowledgement
Not available

Author's Contribution
Not available

Conflict of Interest
Not available

Financial Support
Not available

References

1. Al-Khafaji Basima Rabie. The Effect of Sagebrush, Willow, and Withania Somnifera Extract on the Growth of Some Dermatophytes, Master Thesis, College of Science, Al-Mustansiriya University; c2000.
2. Qutub Hussein Fawzi Taha. Medicinal plants, their cultivation and components, first edition, Mars Publishing House, Riyadh; c1981.
3. Al-Noman, Adeeba Younis Sharif Hamo. The partial effect of some plant extracts on the growth and metabolism of a number of Gram-positive and Gram-negative bacteria, PhD thesis, College of Science, University of Mosul, Iraq; c1998.
4. Al harbi KS, Joshi N, Singh Y, Kazmi Al, Abbasi FA, Alzarea SI, *et al*. Molecular exploration of hidden Pleiotropic activities of azoles on dermatophytes in human tinea Corporis infection. Journal of Medical Mycology, 2022, 101311.
5. Chowdhary A, Singh A, Kaux Ang, Khurana A. The emergence and World-Wide Spread of the Species Trichophyton indetineae causing difficult to treat dermatophytosis: A new challenge in the management of dermatophytosis. Plos Pathogens. 2022;18(9):e1.10795.
6. EL Shaer AHI. Integrated Control of root rot disease of Some legumes M. Sc. Thesis, Faculty of Agriculture, Cairo university, Egypt; c1998.
7. Ellis DH, Davis S, Alexion H, Handke R, Bartley R. Descriptions of Adelaide; c2007.
8. Faway E, Thiry M, Mignon B, Poumay Y. Experimental models of dermatophytosis. In Dermatophyt and Dermatophytoses, 2021, 135-16. Springer, cham.
9. Feng L, Tian X, EL-Kassaby YA, Qiu J, Feng Z, Sun J, Wang T. Predicting suitable habitats of *Melia azedarach* L. in china fusing clatamining, Scientific reports. 2022;12(1):1-10.
10. Forbes BA, Sahm DF, Weissfeld AS. Diagnosis Microbiology 11th ed Mosby Inc. New York, 2002, 1069.
11. Gokats O, Mammadov R, Duru ME, Ozen E, Colak M. Application of extracts from the Poisonous Plant, *Nerium oleander* L., as a Wood Preservative. African, J, of Biotech. 2007;6(17):2000-2003.
12. Harborne JB. Phytochemical Methods. 3rd ed. chapman and Hall, 1998, 512.
13. Janardhan B, Vani G. Clinica mycological Study of dermatophytosis. Int. J Res. Med, Sci. 2017;5:31-39.
14. Jorgensen JH, Pfaller MA, Carroll K, Landary ML, Funke G, Richter S, *et al*. Manual of clinical Microbiology 11th edition-ASM Press; c2015.
15. Kwon Chung KJ, Bennett JE. Medical mycology, Philadelphia, Lea and febinger London, 1992, 105-155.
16. Le Grand A, Wondergem PA, Verfoorte R, Pousset JL. Anti-infections Phytotherapies, of the Tree Savannah of Senegal (West Africa) II Antimicrobail activity of 33 Species. Journal of ethnopharmacology. 1988;22(1):25-31.
17. Mondali NK, Mojumdar A, Chatterje SK, Banerjee A, Datta JK, Gupta S. Antifungal activites and chemical characterizatio of Neem leaf extracts on the growth of Some Selected fungal Species *in vitro* culture medium. J. APP. Sci. Environ Manage. 2009;13(1):49-53.
18. Rios JL, Recio MC, Villar A. Antimicrobial activity of Selected Plants employed in the Spanish Mediterraneanarea. J of Ethnoph. 1987;21:139-152.
19. Shanthi P, Sownthariya C, Sundari UT. HPTLC Profiling and Antibacterial Efficacy of *Melia Azedarach* Linn. Leaf Extracts Against Secondary Bacterial Pathogens of Dermatophytosis. Biomedical & Pharmacology Journal. 2022;15(2):1013-1024.
20. Sharma D, Paul Y. Preliminary and Pharmacological Profile of *Melia azedarach* L. An over view. Journal of Applied Pharmaceutical Science. 2013;3(12):133-138.
21. Duncun OD, Duncun B. A methodological analysis of segregation Index. American Sociological Review. 1955;20:210-7.

How to Cite This Article
Abdul-Jabbar Shanshola NMR, Al-Nuaimi KSH. Inhibition of the growth of the pathogenic fungus *Trichophyton rubrum* by *M. azedarach* leaves. International Journal of Advances in Microbiology Research 2023; 4(1): 11-15. DOI: https://doi.org/