The inhibitory effect of the aqueous extraction of Calotropis procera against some pathogenic fungi

Saba Hassan Alwan1 and Hussein Ali Salim2
Diyala Education Directorate, Ministry of Education, Iraq1
Directorate of Diyala Agriculture, Ministry of Agriculture, Iraq2
E-mail: sab1071981a@gmail.com1
E-mail: h_salim11111@yahoo.com2

Abstract
A laboratory experiment was conducted in the Diyala Agriculture Directorate's plant protection laboratory in November 2018 to investigate the effects of an aqueous extract of the Ashar plant, Calotropis procera, at three different concentrations (10, 20, and 30%) against the pathogenic fungi (Rhizoctonia solani, Aspergillus ochraceus, and Penicillium sp.) in vitro. Findings from the tests showed that the aqueous extract of C. procera had the highest percentage of inhibition against R. solani (86.67%). On the other hand, Penicillium sp. and A. ochraceus had lower percentages of inhibition, at 27.59 and 13.61%, respectively, at the same concentration (30%). The concentration (20%) reached 59.11, 26.44, and 9.68%, respectively, while the concentration (10%) reached 35.11, 24.37, and 6.82%, respectively.

Keywords: Calotropis procera , Rhizoctonia solani, Aspergillus ochraceus, Penicillium sp

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Introduction

Plant diseases cause significant damage during plant growth and development, leading to a reduction in yield and quality of produce [25]. Rhizoctonia solani is an indigenous pathogen found in soil that affects numerous plant families and attacks plants at all phases of growth, resulting in damping off and seed rot either before or after germination [11,29]. One of the most common food-contaminating fungi in nature, Aspergillus ochraceus, is a filamentous fungus that is known to produce mycotoxins such as ochratoxin and citrinin [9]. One of the prevalent fungi found in a variety of environments, including soil, air, and vegetation, is penicillium. It produces mycotoxins [7] and phytopathogens that cause crop rot [20,26]. It is common practice to inhibit phytopathogenic fungi by using synthetic fungicides, and in order to minimise environmental pollution and damage to plants caused by the synthetic fungicides, it is therefore important to reduce the use of synthetic chemicals. As a result, eco-friendly methods must be used to improve crop health and yield, and the most effective replacement for these hazardous chemicals is botanical extracts [24,22]. Calotropis procera is a soft, woody dendritic plant that can grow up to 6 meters tall. It has broad, oval leaves that are green in color and covered in white cotton bristles. Inside, it has purple flowers, hollow green fruits, and brown seeds with silky hair on top [28]. The plant belongs to the class of Dicotyledons, the order Gentianales, the family Asclepiadaceae, and the genus Calotropis [15]. The plant, also known as the huge milkweed, glazes, or apple of Sodom, Burmukh, and Karnaka, is also known as Ashar or Ashr in Arabic. It contains glycosides as well as quasi-alkaloids such as

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calotropin, calactin, clotropaginin, and gigantin, which are considered toxic to living organisms [4]. This plant was used as a treatment in popular medicine, but it is toxic by scientific standards, useful by industrial standards, and it can be used medically by treating it with a chemical or enzyme system to become a therapeutic substance [3]. This study was conducted to test the effectiveness of the aqueous extract of the Ashar plant Calotropis procera against the growth of Aspergillus ochraceus, Penicillium sp., and Rhizoctonia solani in vitro.

Materials and Methods

A lab experiment was conducted at the Plant Protection Laboratory of the Directorate of Diyala Agriculture in 2018. Calotropis procera leaves were collected from a single tree in Baqubah district, Diyala province. The fungi (Aspergillus ochraceus, Rhizoctonia solani, and Penicillium sp.) were obtained from the College of Agriculture, University of Diyala.

Preparation of the aqueous extract of Calotropis procera leaves

After washing the Calotropis procera leaves with water to get rid of dust and suspended impurities, the process involved weighing 20 g of leaves, adding them to 100 ml of sterile distilled water in a beaker for 24 hours, filtering them through a double-layered muslin cloth, and storing them in the refrigerator until use [10.[

Poisoned food test technique

The potato dextrose agar medium (PDA) used in this process was prepared according to [5]. Extract concentrations (10, 20, and 30%) were prepared by adding 10 ml of extract to 90 ml of PDA to get 10%, 20 ml of extract to 80 ml of PDA to get 20%, and 30 ml of extract to 70 ml of PDA to get 30%. The control treatment included only PDA medium. After the medium became solid, agar discs (6 mm) of A. ochraceus, R. solani, and Penicillium sp. from the seven-day-old culture were transferred to petri plates and incubated at 25±2 C for 7 days, according to the poison food technique [18,16]. The tests were performed in three replications, and the diameter of fungus growth was calculated. The percent inhibition of fungal growth was calculated according to [19.[

Inhibition (%) = (1- T/ C) X 100

Where, C = Colony growth of control, T = Colony growth of treatment

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) by using the completely randomized design (CRD) [6 .[

Results and discussion

The results in Table 1 and Figure 1 showed that Calotropis procera concentrations led to a significant increase in the inhibition percentage of Rhizoctonia solani, Aspergillus ochraceus, and Penicillium sp., where a concentration of 30% was significantly superior in the inhibition percentage of mycelial growth, which reached (86.67, 13.61, and 27.59%), followed by concentrations of 20% (59.11, 9.68, and 26.44%) and 10% (35.11, 6.82, and 24.37%), respectively, compared to control (0.0%.(

The aqueous and alcoholic extracts from the Ashar plant are highly effective and inhibit the growth of fungal and bacterial microbes. Their effectiveness may be attributed to the fact that they contain calotorpain protein [13]. In a previous study [2], Aspergillus conidia were treated with different concentrations of Calotropis procera extract (5, 10, and 20%), which led to a significant decrease in the percentage of conidia survival by increasing the extract concentration. [8] stated that the tested concentrations of Ashar leaves were effective in inhibiting the growth of the fungi

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Alternaria sp. and Fusarium sp. [17] reported that the alcoholic aqueous extracts of Ashar flowers inhibited the growth of the fungi Sclerotinia sp. and Geotrichum candidum, as well as three genera of bacterial plant pathogens, Erwini sp., Xanthomonas sp., and Pseudomonas sp. This clearly shows the effectiveness of the Calotropis procera extract as a strong anti-microbial because it contains the protein calotorpain [3]. This is consistent with the study [12], in which Calotropis proceraleaf extract inhibited the germination of the stony bodies of Macrophomina phaseolina, Helminthosporium sp., and Alternaria radicina. [27] stated that the extract of Calotropis procera inhibited the growth of the following fungi, Fusarium oxysporum, Helminthosporium spiciferum, Aspergillus flavus, and Curvularia lunata. This is because the aqueous extract of Calotropis procera kills microbes and stops the growth of the fungus Penicillium sp. It also has a lot of terpenes and alkaloids that have complex biological effects [14]. The variation in the inhibitory activity of the extract on the growth of fungi is due to the nature of the contrast in the active substances and components and their quantities, which affect the inhibitory ability and the nature of the fungus [1]. This is consistent with [21], where it was found that some plant extracts had stimulating effects for some fungi, some of them had an inhibitory effect, while others had no effect. Among the plant extracts that inhibited these fungi (Rhizoctonia solani, Aspergillus ochraceus, and Penicillium sp.) are fenugreek seed extracts [23.[

Conclusion

Calotropis procera extract can inhibit a lot of different types of fungi, including Rhizoctonia solani, Aspergillus ochraceus, and Penicillium sp.; whenever the extract concentration increased, it led to an increase in the rate of fungal inhibition

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Table 1. Effect of different concentrations of *Calotropis procera* extract on inhibition percentages of *Rhizoctonia solani, Aspergillus ochraceus,* and *Penicillium* sp. in vitro

Calotropis procera concentrations	*Rhizoctonia solani*	*Aspergillus ochraceus*	*Penicillium* sp.
Control	0.00	0.00	0.00 d
10%	35.11	6.82	24.37 c
20%	59.11	9.68	26.44 b
30%	86.67	13.61	27.59 a
CD (0.05)	0.01	0.01	0.01

CD = Critical difference

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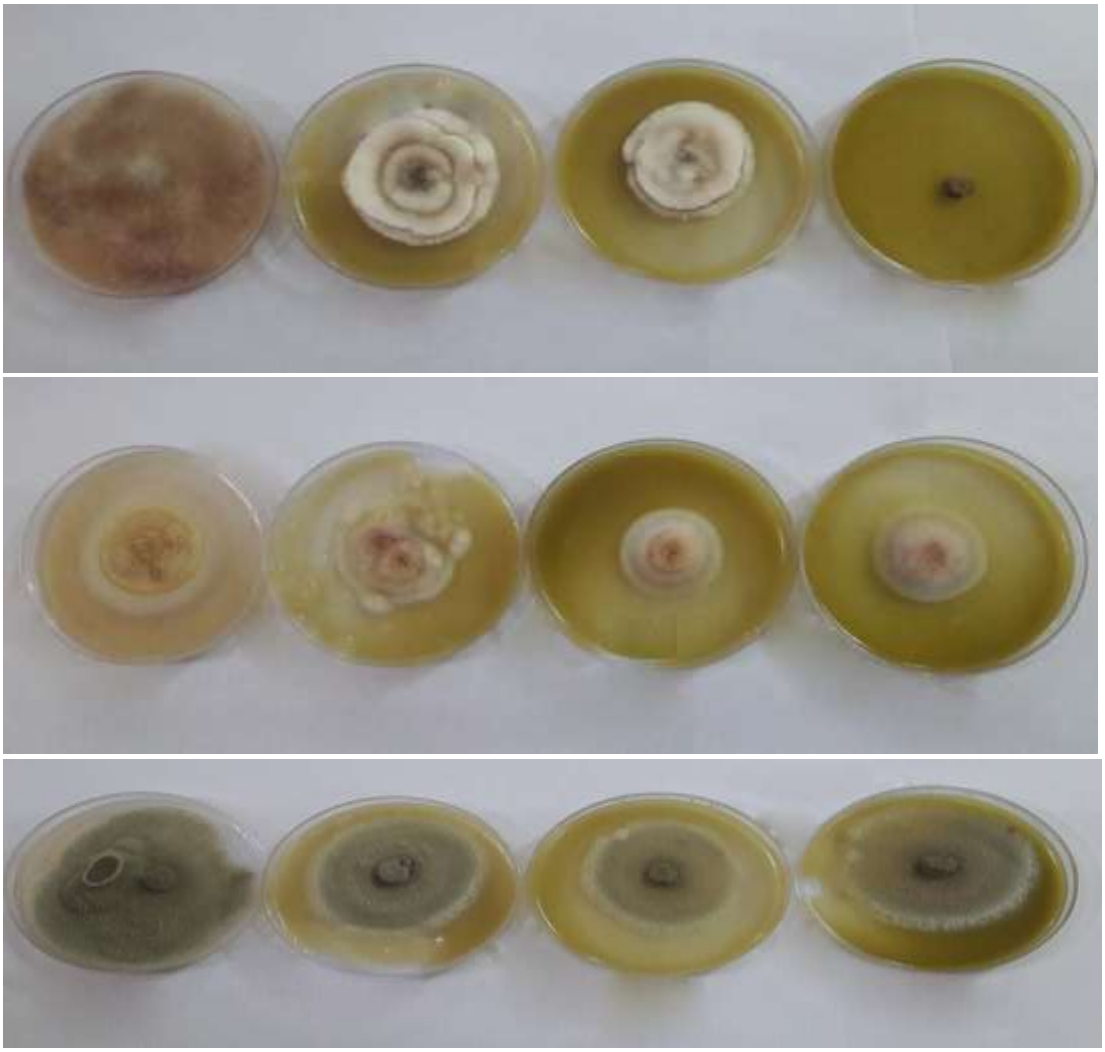

Figure 1. Effect of different concentrations of Calotropis procera extract on inhibition percentages of Rhizoctonia solani, Aspergillus ochraceus, and Penicillium sp. in vitro

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