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Article

The Effect of Some Extracts of Oleander Flowers in Inhibiting the Growth of Some Fungi Causing Onion Rot in Vivo

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Abstract: This research studies the effect of different concentrations of aqueous, Estonian and ethanolic extracts of oleander flowers on the growth of pathogenic fungi of onion plants: *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* sp. All extracts showed high inhibitory effect against the fungi under study, The inhibitory activity varied depending on the solvent and extract concentration and ranged from 8.2% to 100%. The ethanolic extract was the most effective effective in inhibiting the growth of the studied pathogenic fungi compared to the aqueous and acetonic extracts. *Penicillium* sp. was more sensitive to the ethanolic and acetonic extracts compared to *Aspergillus niger* and *Aspergillus flavus*. The concentration of 20 µl/ml for the three extracts achieved high inhibitory activity in the growth of the studied fungi.

Keywords: Plant Extracts, Oleander, *Aspergillus*, *Penicillium*, Onion

Introduction

Onions (*Allium cepa* L.) belong to the Alliaceae family (Andreev, 2003), which was previously called the Amaryllidaceae family, which contains more than 250 genera and about 4200 species (Hassan, 1988). It is considered one of the most important strategic vegetables grown in Iraq after tomatoes and potatoes, as it is considered one of the winter crops that must be provided throughout the year in most countries of the world, including Iraq (Hazra and Som, 2006). Its cultivation is widespread in all governorates of the country, and it is believed that its original homeland is northern Iran or the region extending from Palestine to India from the Asian continent (Matloub et al., 1989).

It has important nutritional and economic benefits because it is rich in carbohydrates and minerals, especially potassium, calcium, phosphorus, etc., and it contains many vitamins and nutrients (USDA, 2015). It has medical importance because it is used as a useful treatment for many diseases (Chevalier ,2010; Barakade and Lokhand,2012) It is recommended to eat onions to prevent cancer because they contain the antioxidant and anti-cancer substance quercetin (Al-Khafaji and Al-Jabouri, 2010) and some secondary organic compounds that contain sulfur as a main element, such as Allyl propyl disulphide (Hassan, 2000; Patil, 1995). Therefore, various countries have sought to pay attention to its cultivation

and increase the areas planted with it to raise the level of production, as the cultivated area in Iraq for the year 2022 of green onions is about 18,391 hectares with an average productivity of 25,572 kg/hectare and a production rate of 47,030 tons (Central Statistical Organization, 2022).

Onions are exposed to storage rot fungi during storage and marketing. About 15 fungi have been recorded to attack onions during storage and transportation, causing a loss of more than 40% of the crop (Aiyer, 1980). Studies have shown that the most important storage diseases are black mold, caused by *Aspergillus niger*, which infects onions in the field and in storage, and blue mold, caused by the fungus *Penicillium* spp. (Tyson, 2004; Agios, 2005; Raju and Naik, 2006)

Aspergillus molds such as *A. flavus* and *A. niger* infect bulbs under hot, humid conditions, while fungi of the genus *Penicillium* sp. cause significant damage to bulbs at low temperatures. *Penicillium* sp. and *Aspergillus* sp. secrete mycotoxins such as ochratoxins or aflatoxins that are harmful to human health (Overy et al. 2005).

Chemical pesticides are used to combat fungal diseases of onions in both the field and the store, because they may cause many problems, including their great danger to the environment and humans as a result of the residual effect of pesticides in food and their bioaccumulation and the formation of strains resistant to these pesticides in addition to their high costs (Paster and Barkai-Golan,2008; Raja, 2014)

Many studies indicate that the use of plant extracts is environmentally safe, has low toxicity to humans, does not accumulate in the environment, and is effective in combating plant pathogens because it contains terpene compounds, phenols, alkaloids, and volatile oils that affect the growth of microbes, fungi, and bacteria that cause diseases (Shivpuri et al.1997; Kagale, 2005; El-Kamali & El-Amir, 2010).

This study aims to know the effectiveness of aqueous, ethanolic and estonic extracts of oleander flowers against the growth of some fungi that are pathogenic to onions in the laboratory.

Methods and Material

Chemical

All chemicals used in present investigation are bring from Hi media and BDH company.

Culture Media: -

a. **Potato dextrose agar (PDA): -** This medium was prepared by dissolving (39) gm of the medium powder in 1 liter of distilled water then mix well to ensure that all powder is dissolved, after which It is heated to a boil and then placed in the autoclave at 120 C^{o} for 20 minutes, and in pressure of 15 pound.

Plant specimens: -

Oleander flowers were collected from a public garden in Dhi Qar Governorate. They were cleaned and washed several times with regular water and then with distilled water. They were left to dry at room temperature, then ground in an electric grinder and stored in a dry, clean, opaque, tightly sealed plastic container in the refrigerator at a temperature of 4 C^{o}. We used them for extraction.

Preparation of aqueous extract:

The method (Ahmed *et al*., 1998) was followed in preparing the aqueous extract by mixing 20 g of oleander powder with 400 ml of Distilled water in a volumetric flask of 1000 ml, then putting the mixture in a shaking water bath at a temperature of 40 C^{o} for 24 hours. After that, the extract was filtered using several layers of medical gauze first, then using milipore filter papers of a type with a diameter of 0.22 μm. The clear liquid was stored in tightly closed containers in the refrigerator at 4°C until use. (Khanzada et al.,2006).

Preparation of the alcoholic extract:

Based on previous studies (Ahmed et al., 1998; Khanzada et al., 2006), 95% ethyl alcohol was chosen to prepare the alcoholic extract in the same way as the aqueous extract.

Preparation of acetone extract:

The same previous method was followed to prepare the acetone extract, replacing distilled water with 70% acetone, according to (Al-Ghanimi, 2007).

Isolation of fungi from onion plants:

Samples of red onions, each weighing 1 kg, showed symptoms of storage rot and the color of their scales changed randomly from a local market in the city of Nasiriyah. The samples were placed in paper bags under laboratory conditions until isolation. The onions were transferred and placed in a Petri dish containing 70% ethanol for three minutes for surface sterilization, then washed three times with sterile distilled water to remove the alcohol and placed on sterile filter paper to get rid of the water. Then they were transferred to dishes containing PDA medium with the antibiotic amended with 250mg L^{-1} chloramphenicol added to it to kill bacteria, at a rate of three pieces per dish and at a rate of 10 dishes at a temperature of 25 - +2 C^{o} for seven days Isolates were purified by transferring a portion of the fungal colony to new Petri dishes containing PDA medium (Hye Ji *et al*., 2018). General and specific taxonomic references were used for the identification for fungal species (Watanabe, 2002; Forbes et al. ,2002; Pitt and Hocking, 2007).

Testing the effect of plant extracts of oleander flowers on the growth of fungi that cause pathogens in onion plants: -

The effectiveness of different extracts of oleander flowers in inhibiting the growth of *Aspergillus niger ,Aspergillus flavus and Penicillium sp. isolated* from onion was tested,This was done by mixing the aqueous, alcoholic and liquid acetone extracts, each separately, with the dissolved PDA medium after it had been sterilized and cooled to a temperature of 50°C, at concentrations of (3, 7, 10, 15, 20) ml of extract/100 ml of the medium, respectively, at a rate of three replicates for each concentration. After the medium had solidified, 6 mm diameter disk of a growing fungal colony was placed on the PDA medium for 7-10 days in a hole of the same diameter in the center of the dish containing one of the aforementioned concentrations, A comparison was made representing:

- Aqueous comparison included a plate containing only PDA medium without adding any other substance.
- Alcoholic comparison included a plate containing PDA medium and ethyl alcohol at the same concentrations mentioned above.
- Acetonic comparison included a plate containing PDA medium and acetone at the same concentrations mentioned above.

The comparison plates were planted with the same fungus and in the same way, then all plates were incubated in the incubator at a temperature of (25-28 °C) for (5-7) days for all isolates. The diameter of the growing colony was measured (the average of two perpendicular diameters) to calculate the inhibition percentage using :(Wanchaitanawong et al., 2005)

The following equation:

$$Percentage\ of\ inhibition = \frac{Colony\ diameter\ average\ in\ comparison\ plates\ -\ Colony\ diameter\ rate\ in\ treatment\ dishes}{Colony\ diameter\ average\ in\ comparison\ plates}$$

Results

The results of the study showed the effectiveness of aqueous, estonian and alcoholic extracts of oleander flowers in inhibiting the mycelial growth of *Penicillium*, *Aspergillus flavus* and *Aspergillus niger* fungi that cause onion rot in the laboratory in an industrial culture medium (PDA).

The results showed that the inhibitory effect of these extracts on the growth of fungal mycelium varied according to the type of fungus, the extract, and the concentration used.

The results show the efficiency of the alcoholic extract, as the average diameters of the fungal colonies of *Aspergillus niger*, *Aspergillus flavus* and *Penicillium sp*. at a concentration of 3% (v/v) reached (60, 55, 48) mm, while the inhibition percentage reached (29.4%, 31.2%, 38.4%), respectively.

At a concentration of 7%, the colony diameters were (30, 42, and 49) mm, and the inhibition percentage was (42.3, 47.5, and 61.5) % respectively. At a concentration of 10%, the colony diameters were (35, 30, and 23) mm, and the inhibition percentage was (58.8, 62.5, and 70.5) %, respectively. At a concentration of 15%, the colony diameters were (23, 15, and 0) mm, and the inhibition percentage was (73, 81.2, and 100) % respectively. At a concentration of 20%, the diameters of all fungi were (0) mm, and the inhibition percentage was 100%, as in (table 1)

In the acetone extract only, it showed inhibitory activity against fungi, as the fungal diameters reached (68, 60, and 50) mm, and the inhibition percentage was (20, 25, and 35.8)%, respectively, at a concentration of 3%, while the colony diameters at a concentration of 10% reached (40, 34, and 31) mm, with an inhibition percentage of (53, 57.5, and 60.2)%, respectively, while the inhibition percentage reached 100% for all fungi studied at a concentration of 20%, as in (table 2) .

In the aqueous extract, the diameters of fungal colonies at a concentration of 3% were (78, 72, and 60) mm, and the inhibition percentage was (8.3, 10, and 23) %, respectively. At a concentration of 10%, the diameters of fungal colonies were (65, 58, 48) mm, and the inhibition percentage was (23.5, 27.5, 38.4) mm, respectively. At a concentration of 20%, the diameters of fungal colonies were (38, 31, and 21) mm, and the inhibition percentage was (55.3, 61.2, 73) %, respectively, as in (table 3).

This is consistent with what was mentioned (Al-Sanidi and Ahmed 2023) that the aqueous extract of oleander leaves at a concentration of 20 ppm was the most effective in inhibiting *Aspergillus niger.(* Al-Sanidi and Shihab,2011) also noted that the aqueous extracts

of oleander leaves had inhibitory effects on *Aspergillus niger*, as the effectiveness of the aqueous extract was (68.26, 58.2 and 55) cm at concentrations of 10, 15 and 20% (weight / volume), respectively..

The type of extract can be arranged according to its effectiveness in inhibiting the fungus as follows: the alcoholic extract>the Estonian extract>the aqueous extract.

As a result of the difference in the dielectric constant of the solvents used in the extraction, which affects the polarity of the solvent and its effectiveness against fungi as a result of the difference in the content of each extract of active substances, this led to the superiority of the alcoholic extract over the acetone and aqueous extracts in preventing the growth of the fungi included in the study. This may be attributed to the fact that oleander extracts contain toxic compounds such as Nerine and Oleandrin, which are toxic substances even if found in small concentrations and which may dissolve in alcohol, thus affecting fungi (Goktas et al., 2007) in addition to the fact that oleander flowers contain resins, flavonoids, tannins and glycosides (Al-Quraishi, 2011).

The results of this study were consistent with previous studies that praised the superiority of alcoholic extracts of oleander flowers, which showed antagonistic activity against a group of skin fungi, including *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum*, and *Aspergillus niger* in a study onducted by (Al-Quraishi, 2011).

This study also matched the study of (Muhammad and Yaqoub 2019), which showed the effectiveness of alcoholic extracts of medicinal plants such as *Coriandrum sativum* against a group of plant pathogenic fungi, *Penicillium*, *Aspergillus*, and *Fusarium oxysporum.*

Table 1. Effect of alcoholic extracts of oleander flowers on the radial growth of the studied fungi

Mean	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium sp.*	Concentration**(µl/ml)**
54.3 (33%)	60 (29.4%)	55 (31.2%)	48 (38.4%)	3
40.3 (50.4%)	49 (42.3%)	42 (47.5%)	30 (61.5)	7
29.3 (64%)	35 (58.8%)	30 (62.5%)	23 (70.5%)	10
12.6 (84.7%)	23 (73%)	15 (81.2%)	0 (100%)	15
0 (100%)	0 (100%)	0 (100%)	0 (100%)	20
81	85	80	78	Control

Average diameter of fungal colony, and inhibition rate (%) in parenthesis

Table 2. Effect of acetone extracts of oleander flowers on the radial growth of the studi ed fungi

Mean	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium sp.*	concentration**(µl/ml)**
59.3 (27%)	68 (20%)	60 (25%)	50 (35.8%)	3
48.7 (40%)	55 (35.3%)	49 (38.7%)	42 (46.1%)	7
35	40	34	31	10

(64.6%)	(53%)	57.5%))	(83.3%)	
25 (69.4%)	34 (60%)	28 (65%)	13 (83.3%)	15
0 (100%)	0 (100%)	0 (100%)	0 (100%)	20
81	85	80	78	Control

Average diameter of fungal colony, and inhibition rate (%) in parenthesis

Table 3. Effect of aqueous extracts of oleander plant diarrhea on the radial growth of the studied fungi

Mean	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium sp.*	concentration**(µl/ml)**
70 (13.7%)	78 (8.2%)	72 (10%)	60 (23%)	3
63.3 (15.2%)	70 (17.6%)	65 (18.7%)	55 (29.4%)	7
57 (30%)	65 (23.5%)	58 (27.5%)	48 (38.4%)	10
43 (46.2%)	51 (40%)	45 (43.7%)	35 (55.1%)	15
30 (63.2%)	38 (55.3%)	31 (61.2%)	21 (73%)	20
81	85	80	78	Control

Average diameter of fungal colony, and inhibition rate (%) in parenthesis

Conclusion

The study highlights the significant antifungal potential of oleander flower extracts against fungi responsible for onion rot, namely Aspergillus niger, Aspergillus flavus, and Penicillium sp. Among the extracts tested, the ethanolic extract exhibited the highest inhibitory activity, achieving complete inhibition (100%) at a concentration of 20 µl/ml for all three fungal species. The acetonic extract followed in effectiveness, while the aqueous extract showed comparatively lower inhibitory activity. These findings emphasize the potential of oleander flower extracts as eco-friendly alternatives to chemical fungicides for managing onion storage fungi, thereby reducing environmental and health risks associated with synthetic chemicals. Future studies can focus on optimizing extraction methods and exploring field applications to enhance their practical usability in agricultural practices.

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