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Use of plant aqueous extract to control fungal diseases

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A B S T R A C T
Medicinal and aromatic plant extracts are considered as an alternative to chemical pesticides to combat plant diseases, including fungal diseases. They were used to protect plant production from diseases caused by fungal, bacterial, viral and nematode organisms that affect various field crops and cause large losses. And reduce losses resulting from infection, whether during the planting season or in the post-harvest stage, in order to keep pace with modern trends in disease control.
Iraq is considered rich in medicinal and aromatic plants and wild plants due to its climate suitable for the growth of these important plants, which are a tremendous source of wealth. More than 250 plant species with medicinal or aromatic uses have been recorded.
Among the medicinal and aromatic plants that are regularly grown in Iraq are basil, mint, lemongrass, garlic, thyme, and others. Some of them grow as ornamental plants or in a wild way, such as oleander, eucalyptus, and datura. All of these plants can use their extracts to combat many plant diseases, in the form of water or alcoholic extracts, or as a powder, to treat either seeds before planting or storage. Or used in the treatment of the root system or as a spray on the vegetative system, whether before or after infection..

Keywords: *plant extracts, fungal diseases,aqueous extracts,fungus*

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INTRODUCTION

One of the reasons for the high percentage of environmental pollution is the use of pesticides to eliminate agricultural pests, and this led to the creation of genetic resistance to many pathogens. From this point of view, many researchers decided to find and develop effective biological means to get rid of pests, including pathogenic fungi, and to achieve another goal, which is not to leave a harmful impact on the environment.

Biocontrol is defined as the use of biological means to combat agricultural pests, such as the use of living organisms or plant extracts belonging to different types, including medical ones, that reduce the amount of pollen of the pathogen to reduce its efficiency in growth and reproduction, directly or indirectly, without leaving a harmful effect on the plant [1].

Plant extracts, essential oils, and salts have an anti-plant disease effect. Studies indicate that their effect is either by not enabling fungal spores to stick to the surface of the paper, or it may lead to killing the fungus and inhibiting it during spore germination and growth, and thus preventing the fungus from settling in the plant tissue. For example, many Among the compounds present in the aqueous extract of the ivy plant Hedrahelix had an insecticidal effect on the spores of the fungus Venturiainainaequalis.

Plant extracts were used to control the infection of fungi that cause storage rots, such as aqueous extracts of algae Hypogyminaphysodes, Ramalinafarinacea, which inhibited the growth of Aspergillusflavus by 70-80%, (60-65%) Tentex-T.

Cinnamon oil and garlic cloves prevent infection Chaetomiumindicum and Curvulariapallesecens in maize [2]. The oil of young citrus medica leaves inhibited the growth of Aspergillus runner and Penicillium on peanut seeds [3]. The effectiveness of the extracts of the same plant may differ according to the solvent used in the extraction or according to the plant part used and also according to the age stage of the plant part used, or the effectiveness of the plant extracts may differ according to the stage of the plant treated with the extract [4].

Mint is one of the herbal plants grown in Iraq and contains volatile oil rich in menthol, bethine and tannin. The part used when extracting is the leaves and flowering tops. Peppermint extract affects the growth of Aspergillusnidulans and E. coli[5].

Pomegranate peels are very distinctive among plant extracts, as they contain four types of alkaloids: Pelletierine, which is called Punicine, Isopelletrine, and Ethyl pelletierine, and the alkaloid Pseudo pelletierine, which is called Methylgrantanine [6].

The Datura plant is one of the medicinal plants that contain hyoscyamine, atropine, and scopola as a painkiller, anesthetic, and hypnotic. It is used in the fight against (A. flavus, B. theobromae, F.oxysporum).

As for the Nerium plant, it is considered one of the important plants as a medicinal crop, and it is an ornamental plant. The leaves of the oleander contain the substances nibrin, nerianthin, and pandarin, and medicines that work to strengthen the heart muscles are made from it. The extracts of this plant are used to inhibit the mycelium and germination of spores of many fungi that contaminate seeds (such as maize), namely (Alternariaalternata, F.monilliforme, Cochliobolus lunatus, Aspergillusflavus, Rhizopusstolonifer). The active substances can be extracted by warm water and alcoholic solvents.

The leaves of camphor trees are among the important parts because they contain 1.5 to 3.5% volatile oils, and the main compound in these leaves is cineole, which represents about 54 to 95%. The amount of oils varies according to the age of the leaves, and the oil is produced within the first four hours of extraction. The extract is rich in flavonoids, triterpenes. The camphor extract is used against Gram-negative bacteria and fungi (R.solani or F.solani).

As for the garlic extract, it works to resist many bacterial and fungal plant pathogens, especially those that affect the vegetative system, such as (Pseudomanasphaseclica, Xanthomonas sp., Puriculariaoryzae, Colletrotrichum sp., Pseudopernosporncubnusis, Moniliafructucola).

2. EXTRACTION METHODS:

2.1. Preparation of aqueous extracts:

The method of Harborne [7] is usually followed to prepare the aqueous extract, whereby 20 g of each dry sample of the different plant parts is taken and placed in a 500 ml conical flask and a certain amount of distilled water is added to it at a temperature of 20-25 °C and the volume is completed to 200 ml, and placed with a device (Horizontal Shaker) (GFL Model 3015) for half an hour at a medium speed. The samples are left to settle for an hour, then filtered with three layers of gauze to separate solid plankton, then sedimentation is carried out using a centrifuge at a speed of 3000 rpm for 15 minutes to separate small plankton. The dry matter is used in the preparation of different concentrations of extracts.

2.2. Reagents used to identify types or groups of secondary compounds in aqueous extracts:

1. Ferric chloride solution: to determine the presence of tannins and phenols [7].
2. Lead acetate solution: to determine the presence of tannins [8].
3. Meyer's reagent: for the detection of pan-alkaloids [7].
4. Fehlink's reagent: to determine the presence of glycosides [9].
5. Iron and potassium cyanide solution: to determine the presence of phenols [7]

2.3. Isolation and identification of fungi from infected plant fruits:

Small pieces of infected fruits that showed symptoms of the disease are taken, each with a diameter of 0.5 cm, then sterilized with sodium hypochlorite solution at a concentration of 3% for one minute, then washed with sterile water to remove the effect of the sterile solution three times, dried on sterile filter paper, then placed in Petri dishes containing PDA medium Three pieces per plate. The plates are incubated at a temperature of 25 °C for seven days. After the end of the incubation period, the fungus isolates are purified using PDA medium. They are diagnosed based on the phenotypic characteristics of the colonies and microscopic characteristics. Taxonomic keys are used for this purpose [10,11].

2.4. How to calculate the effect of aqueous extract on mushroom growth rates:

To calculate the effect of the aqueous extract on the radial growth of the fungus, PDA was distributed in six conical flasks of 250 ml, with 120 ml of medium for each flask, and after sterilization and low temperature to pre-solidification, the aqueous extract of the plant used in the experiment was added in different quantities to five glass flasks to obtain The concentrations are different and the sixth flask is left without addition as a control treatment. The pH of the culture media was adjusted to 6.5 under sterile conditions, then the media was poured into glass dishes, each with a diameter of 9 cm, and the center of each dish was inoculated with a disc of 10 mm in diameter taken from the edge of a fungal colony growing on the PDA medium at the age of four days, with three replications for each fungus at each concentration. In addition to the control treatment, the dishes were incubated at a temperature of 25 °C for seven days. The results are recorded at the end of the incubation period by taking the average of two orthogonal diameters passing through the center of the disc and calculating the inhibition percentage according to the following equation:
Inhibition rate = (growth in control-growth in treatment/growth in control) x 100[12].

2.5. Storage experience and how to treat fruits with aqueous extract:

The treatment was adopted by immersion method for the fruits. After selecting a plant and making sure that it is free of any symptoms of infection, at the rate of half a kilogram for each treatment, as for the uninjured fruit treatments, the fruits are contaminated with fungus suspension at a concentration of $10^{3}3.7\times$, then each of them is placed inside three layers of used paper for the purpose of preserving foodstuffs that are sold in shops. Before that, the fruits are treated with extract concentrations separately inside sterile nylon bags for ten minutes with shaking to ensure that the extract completely covers all the fruits, in addition to conducting a control treatment by treating the fruits with fungal suspension only and other control without contamination, with three replicates for each treatment. The paper-wrapped fruits are all stored at the laboratory temperature for one week at the laboratory temperature, after which the results are recorded by calculating the percentage of infection for the fruits and for all treatments as follows: [13]

Infection rate = number of infected fruits / total number of fruits * 100

As for the wounded fruits, the same previous steps were taken, with the exception of making cuts of 5 mm in length and depth in each fruit, at the rate of three cuts for each treatment, using a sharp and sterile blade. At the end of the storage period, small pieces of fruits that showed symptoms of infection are taken, and after sterilization, they are planted in a petri dish containing a PDA and incubated at a temperature of 25 C for a period of seven days for the purpose of confirming infection with the fungus [12].

2.6. Statistical analysis:

The results of the experiments are analyzed according to the factorial experiment model with completely randomized design. The Least Significant Difference (L.S.D.) test may be used below the level of 0.05 to show the significance of the results [14].

3. CONCLUSION

Fungal diseases that affect plants remain a cause of great crop losses, despite the use of fungicides. Recently, different mechanisms of action have been identified in the microbial biocontrol of fungal plant diseases including competition for space or nutrients, production of antifungal metabolites, secretion of hydrolytic enzymes such as chitinases and glucans, uses of plant extracts. However, this approach is still in its infancy, due to prohibitive production costs.

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