Isolation and identification of the microorganisms associated with some nymphs and adults of the dubas insect (*Ommatissus lybicus*) and evaluation of their effectiveness in the laboratory

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Abstract

The study aimed to isolate and diagnose the fungi associated with the Dubas insect and test their effectiveness in resisting this insect under laboratory conditions**.** The pest is feeding on leves of date plam tree. That couse dameg to dates. The results showed the presence of fungi associated with the samples of Dubas nymphs and adults in all samples included in the survey, as there was a difference in the percentage of frequency and presence of the fungi, as the *Aspergillus* fungus excelled with the highest percentage of appearance and frequency of 57.14 and 48.13%, respectively. The results of the pathogenicity test of the fungi isolated from Adults and nymphs of Dubas under laboratory conditions, the T2 (*Penicillium* sp.) fungus isolate in sample No. 1 was distinguished by giving it a high mortality rate of 6.58% , followed by the T1 (*Aspergillus* sp.) fungus isolate in the same sample, where its rate reached 4.49% compared to the control treatment in which only distilled water was used, as its rate reached 0.75%. The results of the identification of fungal isolates pathogenic to Dubas insects showed that 4 fungal isolates excelled in the pathogenicity test in the laboratory, and according to the taxonomic keys, they belonged to the species *Aspergillus funigatus*, *A. versicolor*, *Penicillium janthinellum*, and *P. amrantiogriseum*.

Keywords: Dubas, entomopathogenic fungi, Ommatissus lybicus

Introduction

The date palm is economically important fruit trees and the backbone of agricultural activity, as many different types of trees, vegetable crops, and fodder grow under its shade (1). Its fruits are used as a food substance rich in sugars and other minerals in many countries of the world; in addition to being in the manufacture of drinks, it also uses its other parts, such as the stem and fronds, in the manufacture of fences and some local equipment. It is also grown as windbreaks on the edges of various farms, and it is considered one of the means of combating desertification in many Arab countries because it protects the plants grown with or under it (16,32 and15).

Palm trees are exposed to many agricultural pests, which are accompanied by a decrease and deterioration in production, Dubas insect , *Ommatissus lybicus* (Hemiptera: Tropiduchidae) is one of the primaries of these pests, which infects all types of palm trees and is found in all areas of its cultivation .

The infestation is concentrated in orchards near rivers planted closely together (3) . Male and Female of pest feeding on the leaves (14) The insect spreads in Iraq, the United Arab Emirates, the Sultanate of Oman, the Kingdom of Saudi Arabia, Libya, Algeria, Egypt and Iran(4) . Its great harm comes through its absorption of plant sap from fronds, stems, and fruits, in addition to the damage resulting from the molasses substance on which many saprophytic fungi grow, causing the weakness of palm trees and their lack of production(15). Palm trees may not produce in the case of severe infection, and trees may die if the infection continues for several years. without control, the economic losses resulting from its infestation were recorded in many countries, as it was able, within a month and a half, to eliminate nearly a quarter of a million palm trees in Hadhramaut in Yemen (29). Palm bulletins indicate that the Iraqi Dates Authority adopted the first control of this insect in the years 1936, 1935, and 1934 were used at that time mixture of nicotine powder, Nora, and ash (6). After that, chemical pesticides such as DDT, heptachlor, dia-xenon, and Diptrex were used (1). Alternative method against dubas insect are needed to avoid the harmful effects of chemical control and the extensive use of pesticides include the development of resistance to many types of pesticides, the harmful effect on beneficial insects, especially biological enemies (parasitoids and predators), which led to reduce the environmental effect of a insecticide (10).

It has become necessary to have safe methods for controlling dubas insect. Biological control agents represented by predators, parasites, and pathogens have proven highly effective in resisting the Dubas pest (20,18 and 21). (11) were able to isolate Types of Penicillium, Aspergillus, and other fungi from apricot, whitefly, and Dubs insects, and they proved effective when used against nymphs and adults of these insects (7). We also isolated the fungi associated with the white scale insect , Alternaria, Cladosporium,Fusarium,Helminthosporim , and Phoma and tested their pathogenicity on palm fronds. Dates and the white scale insect.

The objectives of this study are to isolate the fungi associated with the Dubas insect and test their effectiveness in resisting this insect under laboratory conditions.

Materials and methods

Isolation and identification of fungi associated with nymphs and adults of the *Ommatissus lybicus* insect.

Samples were collected randomly from the field identified in the Al-Wand area of Karbala Governorate on 10/25/2022 and brought to the Bio resistance Laboratory of the College of Agriculture, University of Karbala, Department of Plant Protection. The samples included leaves from the field of data palms infested with insect stages, and the samples were taken from different directions of trees. The palm trees included four directions (north, south, east, and west), representing the Dubas insect in the nymph and adult stages. To conduct the process of isolating the fungi associated with and contaminated with the Dubas insect, the laboratory work began by preparing the culture medium P D A (Potato Dextrose Agar) by taking 39 grams of the prepared culture medium. It was dissolved in half a liter of distilled water and complete the volume to 1 liter, then sterilized the culture medium with a steam sterilizer at a temperature of 121°C and a pressure of 15 pounds. for 20 minutes, the sterile medium was cooled and transferred to the isolation room. The culture medium was poured into sterile Petri dishes and left to solidify. The dishes were then stored in the refrigerator, the culture mediumN.A.(Nutrient Agar) was also prepared by taking 28 grams of the culture medium per 1 liter, according to the previous steps. The process of isolating the nymphs and adults of the Dubas insect was then carried out by sterilizing the insects with 70% ethyl alcohol for 5 seconds and then washing them with sterile distilled water, in addition to sterilizing the tools used in the isolation process, Dubas nymphs and adults whose length was less than 1 cm were selected. In addition, honeydew swabs were taken to detect fungi and bacteria growing on them. They were placed in sterile dishes containing the P D A culture medium and those containing the culture medium. N.A. Prepared in advance, with the same steps, the samples were also planted without sterilization, with four nymphs or adults per dish. The dishes were then placed in sterile polyethylene bags and incubated at ±25°C for 2-5 days. After the end of the incubation period, the isolates were purified using the single-spore method. The method of marking on a number of dishes using a sterile circular needle (Loop) in Petri dishes containing the nutrient culture medium P D A. Then the germinated colony was taken from the single spore and transferred to new dishes including the same medium and then incubated at a temperature of +25 for 5-7 days. Yeasts and bacteria were also purified using the planning method above.

Fungal colonies, bacteria, and yeasts growing in dishes were phenotypically identified to the genus level using a compound light microscope based on the phenotypic characteristics, including the color and shape of the colonies and the method and speed of their growth mentioned in the approved taxonomic keys (12, 31, 25and28). The occurrence and frequency of fungal isolates were calculated according to the following equations:

Percentage of appearance of fungal isolates

$$= \frac{\text{the fungi in which the samples appeared number}}{\text{the total samples number}} \times 100$$ (27).

Percentage frequency of fungal isolates = $\frac{\text{number of isolates per fungus}}{\text{number of samples per college}} \times 100$ (30).

Testing the pathogenicity of fungi isolated from adults and nymphs of Dubas against them under laboratory conditions

The pathogenicity of ten fungal isolates obtained from isolation from nymphs and adults of the Dubas insect was tested. It included six isolates of the *Aspergillus* fungus, namely T1, T4, T6, T8, T9, and T12, two isolates of the *Penicillium* fungus, namely T2 and T14, one isolate of the *Rhizopus* fungus, which is T5, an isolate of the Alternaria fungus, which is T7, in addition to two bacterial isolates, namely T3 and T11 and two yeast isolates, namely T10 and T13. The experiment included 14 treatments. The test was carried out by bringing samples of palm fronds from the field infected with the Dubas insect. Numbers of Dubas adults were taken 13 adults and placed in plastic containers of 0.5 kg, with three replicates for each treatment. The fungal isolates, bacteria, and yeasts were then activated by growing them on the previously mentioned culture medium, after which they were left in the incubator for 5 days. Then, a suspension of the spores of each fungus was prepared in each dish by adding 10 ml of sterile distilled water to each dish and shaking it well, then adding it to 90 ml of sterile distilled water. It was prepared five 10 ml test tubes containing 9 ml of sterile distilled water. Then, 1 ml of the spore suspension was added to the first tube. It was then shaken, and 1 ml was taken from it to the second tube, and so on for the rest of the tubes to make the required dilutions. The particles in the spore suspension were calculated using a Homecytometer slide. After counting the cells and shaking them well, the spore suspension was sprayed at a concentration of 1 x $^{6}10$ on the plastic containers containing the Dubas insects for all isolates at a rate of 3 replicates for each treatment. Bacteria were added similarly, at a 1 x 10 ^{8} concentration. The results were taken over two weeks, at a reading rate for every two days, by measuring the percentage of mortalities of nymphs and adults.

. Dubas insect according to the following equation:

$$\text{Corrected depreciation percentage} = \frac{\%\text{Transaction in Damage} - \%\text{Comparison in Damage}}{100 - \%\text{Comparison in Damage}} \times 100 \quad (5).$$

Identification of effective fungal isolates to the species level

The fungi that proved effective on nymphs and adults of Dubas insects during the previous experiment were identified to the species level by activating the fungal isolates and growing them on P.D.A. culture medium. Glass slides were prepared from each fungal isolate and examined carefully using a compound light microscope to identify their microscopic characteristics in terms of the nature and shape of the mycelium, the conidiophore, the method of carrying spores, and the branches of the sauropod, following the standards followed in the approved taxonomic keys (25 and 28).

Statistical design and analysis

A completely randomized complete design (CRD) was used, the SAS program was used in statistical analysis, and the least significant difference (LSD) test was used at the probability level of 0.01 (9).

Results and discussion

The results in (Table 1 and Table 2) showed the appearance and frequency of fungi, bacteria, and yeasts associated with the samples of nymphs and adults of Dubas in all samples included in the survey, as there was a difference in their frequency and appearance, as the *Aspergillus* fungus had the highest appearance and frequency in sample (3) (Figure 1) They reached 57.14 and 53.57%, respectively, followed by bacteria in sample (4), with an occurrence rate and frequency of 33.33 and 39.13%, respectively. As for the rest of the

isolates, their appearance rate ranged between 1.56 - 48.74%, and their frequency rate ranged between 0.0 - 51.61%, respectively. Yeast, bacteria, *Alternaria* fungi, *Penicillium* fungi, and Rhizopus fungi, and the reason for this difference in the percentage of appearance and frequency of fungi according to the regions from which the samples were collected is due to the difference in the fungi's production of reproductive units and the speed of their spread, as well as the difference in environmental conditions suitable for them, especially the temperature and humidity suitable for them. ~~in~~ in addition, the role of the widespread plant host in the sample collection areas, which plays a major role in increasing or decreasing the activity of the dominant fungal species in the area from which the samples were collected (22). and these results are consistent with the study conducted by(17) who proved the presence of fungi in various agricultural environments. Other factors that increase the presence of the Dubas insect are the density of palm trees, the availability of appropriate humidity, and the orchard's proximity to rivers, which helped the insect to be present in (2).

Table 1. The percentage of appearance of fungi, bacteria, and yeasts associated with the *Ommatissus lybicus*.

No. of samples / **Name of fungus**	**(%)** The percentage of appearance				
	1	**2**	**3**	**4**	**Means**
Aspergillus sp.	47.22	40.62	57.14	50.00	48.74
Pencillum sp.	14.7	6.25	2.77	5.55	5.42
Alternaria sp.	8.33	0	0	0	2.08
Saccharomyces sp.	25.00	0	7.14	0	8.08
Bacillus sp.	5.55	21.87	25.00	33.33	21.43
Rhizopus sp.	0	6.25	0	0	1.56

*The numbers represent the sample collection areas

Table 2. The percentage of frequency of fungi, bacteria, and yeast isolates accompanying nymphs and adults of the Dubas insect

Samples / **Fungus**	The percentage of frequency			
	1	2	3	4
Aspergillus sp.	51.61	45.00	53.57	38.98
Pencillum sp.	30.39	4.67	12.40	10.35
Alternaria sp.	6.45	0.00	0.00	0.00
Saccharomyces sp.	11.55	12.85	21.13	5.10
Bacillus sp.	6.45	26.66	12.90	39.13
Rhizopus sp.	0.00	10.82	0.00	6.44

*The numbers represent the sample collection areas.

Figure 1. Fungal isolates and associated with bacteria appeared during the isolation process from *Ommatissus lybicus* nymphs and adults.

Testing the pathogenicity of fungi isolated from Dubas adults and nymphs under laboratory conditions

The present findings show that (Table 3) the fungal isolate T2 (*Penicillium* sp.) achieved ~~a~~ the highest mortality percentages of adult Dubas insects, as its mortality reached 6.58%, followed by the fungal isolate T1 (*Aspergillus* sp.), which recorded a mortality rate of 4.49%. The fungal isolate T12 (*Aspergillus* sp.)and T14 (*Penicillium* sp.) had a mortality rate of 4.24 and 3.74%, respectively . As for the rest of the fungal isolates, the mortality rate was 3.49 and 3.33%, respectively, compared with control(distilled water), which amounted to 0.75%, compared to other treatments in which insect-pathogenic fungi were used.

The results indicate that significant differences between the fungal isolates tested in terms of their effect on the adults of the Dubas insect under laboratory conditions, through their effect on the adults of the Dubas insect. The secretion of some fungi, enzymes, and mycotoxins affect adult Dubas, which is consistent with previous studies (8). The reason is also due to the rapid growth and multiplication of fungi and their effect on the insect through their secretion of mycotoxins and the formation of fungal spores and their ability to penetrate the insect's body wall due to the chitin enzyme that hydrolyzes the body wall's chitin and attack the mycelia of the fungus into the insect's body cavity and the rest of the body's organs and rob it of its nutritional contents.

Table 3. Percentage of-mortalityof Dubas nymphs and adults treated with fungi and some associated with bacteria under laboratory conditions

S	Treatments	(%) First	(%) Second	(%) Third	(%) Fourth	Mean of Treat
1	T1 (*Aspergillus* sp.)	2.33	4.33	5.66	5.66	4.49
2	T2 (*Penicillium* sp.)	5.00	5.33	8.00	8.00	6.58
3	T3 (*Bacillus* sp.)	1.66	3.00	3.33	3.33	2.83
4	T4 (*Aspergillus* sp.)	1.66	3.00	4.00	4.66	3.33
5	T5 (*Rhizopus* sp.)	1.00	2.33	2.66	2.66	2.16
6	T6 (*Aspergillus* sp.)	1.33	3.00	4.66	5.00	3.49
7	T7 (*Alternaria* sp.)	0.33	0.33	0.33	1.00	0.49
8	T8 (*Aspergillus* sp.)	0.33	2.66	3.00	4.00	2.49
9	T9 (*Aspergillus* sp.)	1.33	2.66	3.00	3.00	2.49
10	(T10 *Saccharomyces* sp.)	1.33	2.66	3.00	3.33	2.58
11	T11 (*Bacillus* sp.)	1.00	1.33	2.00	2.33	1.66
12	T12 (*Aspergillus* sp.)	2.66	4.33	4.33	5.66	4.24
13	(T13 *Saccharomyces* sp.)	1.00	2.66	2.66	2.66	2.24
14	(T14 *Penicillum* sp.)	1.00	3.66	5.00	5.33	3.74
15	Control	0.00	1.00	1.00	1.00	0.75
	L S D %1	**Treatment 0.46**	**Times 0.69**	**Interactions 1.05**		

Identification of fungal isolates pathogenic to Dubas insects to the species level

The results (Table 4) showed the identification of 4 fungal isolates associated with Dubas insects that excelled in the laboratory pathogenicity test and were given a high mortality rate, namely T1, T2, T12, and T14 (Figure 2) based on the phenotypic characteristics including the shape and color of the colony and the method and speed of its growth for the fungal isolates growing on the medium. PDA culture and microscopic characteristics in terms of spore shape and hyphens. Many types of fungi pathogenic to insects have been identified (23). The fungus Clonostachys rosea has been recorded, proving its pathogenic effectiveness against insects.

Table 4. Phenotypic and microscopic taxonomic indicators of the identification fungal species

S	Symbol of isolation	Name of Fungus	Taxonomic characters
1	T1	*Aspergillus fumigatus*	Colony color: white at first, then turns grey Sporophore: transparent, smooth The gizzard is a small spherical Spores: oval Phialides: Uniseriate These results are consistent with findings reported by Hussein,et al.2022 (19).
2	T2	*Pencillum janthinellum*	Colony color: white, then changing from green to transparent orange or yellow Conidiophores: grey-green and smooth Conidia: greenish-gray spherical Biverticillate or Monoverticillate and these results are consistent with findings reported by Pitt, J.I., and Hocking A.D.2009 (26)
3	T12	*Aspergillus versicolor*	The color of the colony is white at first and then turns yellowish-green Conidiophores: transparent or slightly pigmented with smooth walls Conidia: spherical in shape, brown in colour Vesicles: semi-spherical to oval Phialides: Biseriate and originate in the upper section of Metulae The results agreed with Chandra et al 2022.(13).
4	T14	*P. aurantiogriseum*	Colony color: dark green Conidiophores: grey-green and smooth Conidia: spherical with smooth walls Terverticillate or Biverticillate. These results are consistent with Moslem, et al. 2010 (24).

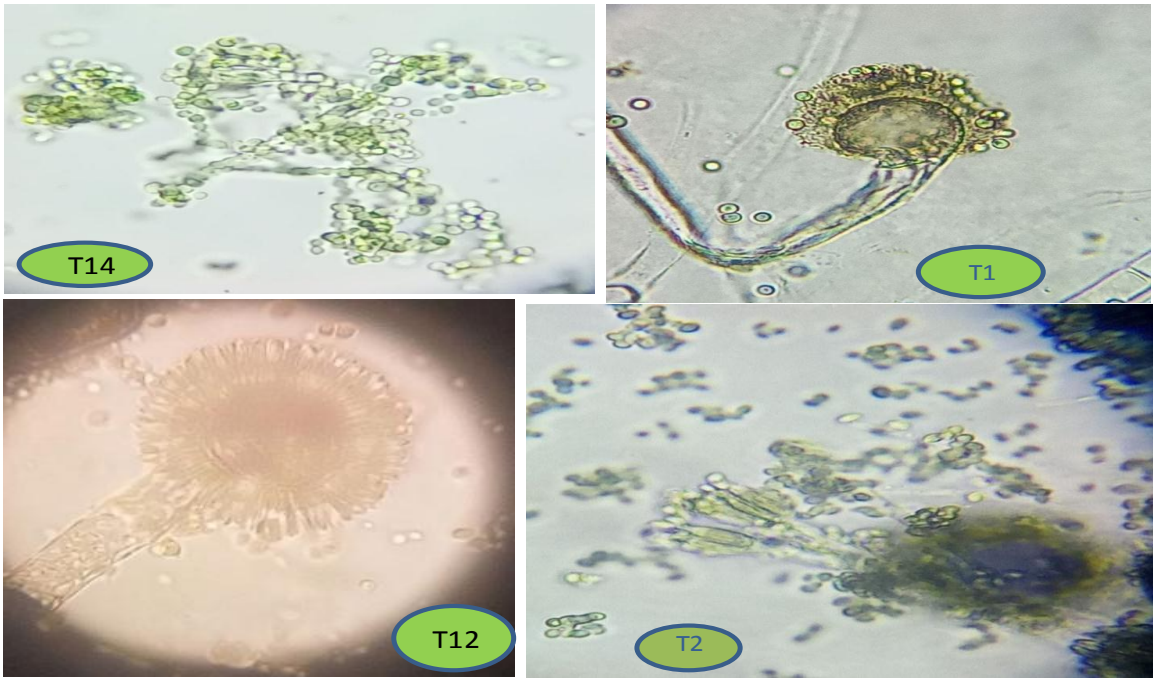

Figure 2. Microscopic characteristics of some fungal isolates T14 (*P. aurantiogriseum*), T1 (*Aspergillus fumigatus*), T12 (*Aspergillus versicolor*), T2 (*Aspergillus versicolor*).

Conclusion

Based on the results obtained from the current study, Aspergillus fungi were superior, with the highest percentage of presence and frequency reaching 57.14 and 48.13%, respectively. Also, 4 fungal isolates excelled in the laboratory pathogenicity experiment: *Aspergillus fumigatus*, *Aspergillus versicolor*, *Penicillum janthinellum*, and *P. aurantiogriseum.*

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Conflict of interest

The authors have no conflict of interest.

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