



EVALUATION OF THE EFFECT OF AL-JAAFARI EXTRACTS *TARGETES ERECTA* ON THE GROWTH, VIABILITY AND SPORES FORMATION OF FUNGUS *PAECILOMYCES LILACINUS*

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Abstract

The aim of this study was to investigate the effect of the aqueous and alcohol extracts of *T. erecta* in the daily growth rate, viability and forming the spores of fungus *P. lilacinus*. The results of this study that we obtained to show that it is possible or impossible to use this fungus with the extract of Al- Jaafari plant in the integrated resistance programs of pathological complexes. The results showed that the extract with both aqueous and alcohol had a negative effect on the growth of the fungus under study and significantly different from the comparison treatment, but did not stop its growth completely even in the highest concentrations that used, where the daily growth rate of the fungus was 0.62 and 0.67 cm when treated with aqueous and alcohol extracts respectively, compared to the comparison treatment (1 cm) and results showed also the superiority of the alcoholic extract over the aqueous extract in its effect on the viability of spores, when treated with aqueous extract, the average number of colonies of fungi was 28 colonies, while 24.6 colonies with alcohol extract with significant difference between the effect of two extracts and the effect increased with the increased concentration that we used. The results showed also that the effect of Al-Jaafari extract in spore germination and a significant difference from the comparison treatment. The percentage of inhibition was directly proportional to the concentration, where the concentration of 500 ppm recorded an inhibitory rate of 11.4% and 14.3% of the aqueous and alcohol extracts respectively. This percentage increased for both extracts with an increase of concentration it reaches 55.7% and 57% for aqueous and alcohol extracts respectively at 2000 ppm. But did not stop its growth completely even in the highest concentrations that we used.

Introduction

Paecilomyces lilacinus is a fungus that is endemic to the soil and has a wide spread. It can be isolated from cultivated and uncultivated soils, estuaries, sewage and deserts. It has ability to parasitism on eggs and females of different types of nematodes. It is found also in a root region in many crops (Anderson *et al.*, 1995; Samson, 1975) The fungus can tolerate a wide range of temperatures ranging from 15 to 30 °c and wide range of pH the optimum temperatures for its growth ranges from 25 to 30°C (Jatal, 1985). It belongs to the division Deutromycota and class hyphomycetes, which is near to Penicillium (Samson, 1975). This fungus has been widely used in biocontrol programs to control various agricultural

pests such as insects, mites (Wakil *et al.*, 2012; Sanjaya *et al.*, 2016) and various types of nematodes, the most famous of these are the *Meloidogyne incognita*, *Meloidogyne javanica* and Heterodera vesicles (Jamali; Ghasemi, 2016; Musksood; Tabreiz, 2010; Baheti *et al.*, 2015) Due to the good results achieved to control some fungus in most biological control programs, it has been produced and commercialized as a biopharmaceutical under several trade names, including Bioact WG, which is used in the control of nematodes worldwide, and the US-based biochemist Melocon WG (Kiewnick; Sikora, 2006). This genus has many species, some of which have sexual phase belonging to the genus Talaromyces of the Division of fungus Ascomycota under the order of Eurotiales and the family of Trichocomaceae and other species possess the stages of sex belonging to the races

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of the species *Thermoascus* and *Byssochlamys* of the same order and family, Carrying cells called Phialids and thus called the Phialoconidia and the cells are generated with a bulging base and a long-necked with chains in a sequence of basic basipetal succession, that is, the most recent cell is in the base and the oldest is in the top, The most common species of the genus are *Paecilomyces lilacinus*, *Paecilomyces marquandii* (Webster; Weber, 2007). The plant is used in this research to obtain the extract from it is called *Tagetes erecta*, which returns to the order of Asterales and the Asteraceae family of floral plants. The plant is known in many European names as al-Jaafari and al-Qatifah and foreign names such as Marigold, Abuba, Omosumo and other names. The original habitat of this plant is north and south America, specifically Mexico, where it is often called Mexican marigold, Extensive in many Asian countries including India, China, Nepal. etc (Halim, 2007; Dasgupta *et al.*, 2012). The length of the plant varies depending on the species and ranges between 50 - 100 cm and the colors vary between yellow, gold and orange. This genus includes large numbers of species, the most famous of which are three types: patula, erecta. menuta There are many studies on the use of this plant extract in the resistance of fungi, including against the fungus *Penicillium digitatum* and *Botrytis cinerea*, where the use of the Al- Jaafari plant *Tagetes patula* for this purpose, one study pointed out that one of the essential compounds responsible for the anti-Jaafari extract is essential oils containing piperitenone and piperitone and chemical compounds found within the components of these oils, which had a significant impact on these cellular changes. (Romagnoli *et al.*, 2005). She also noted that the fungal hypha showed traces of necrotic, necrosis appear after 24 hours of treatment, The smell of this plant expel insects and some small animals (Vijay, 2003; Ikkon, 2007; Idarraga, 2011).

Materials and Methods

Formation of Al - Jaafari plants for extract

The plant of Al - Jaafari species *Tagetes erecta* was obtained from the plant nursery of the Collage of Agriculture - Wasit University. The plants was thoroughly cleaned and washed from the suspended soil. It was placed in plastic trays at room temperature (25 ± 2) for drying with continual fluxing. After the plant dried completely, it was grinded with an electric mill to obtain Al-Jaafari powder, then put the powder inside plastic case and kept it in dry conditions until extraction is carried out (Kafaji, 2005)

Prepare aqueous extract

This extract was prepared by taking 100 g dry powder

of al-Jaafari plant, It was mixed with 400 ml of distilled water in a 1000 mL flask and left it for 24 hours at laboratory temperature. The mixture was filtered by using several layers of medical gauze to remove the suspended materials, then centrifuged by using a centrifuge at 3000 rpm for 10 minutes. The filtering extract was filtered by using a 0.2 μm millipore filter for obtained a clear solution, the extract was kept in the refrigerator at 4^oc until use.

Prepare alcoholic extract

This extract was prepared according to the method of (Anessiny; Peres,1993) by taking 100 g of dry powder from Al- Jaafari plant, placed in a 1000 mL glass flask, adding 500 ml of ethyl alcohol at 70% concentration and leaving it for 24 hours at room temperature. The mixture was centerifuged by using centerifuge system 3000 rpm / mint for 15 minutes, then use the filter paper 1.N o what man to purified the solution from sediments, then the solution was evaporated by using the air drier at a temperature 37p until obtained concentrated solution and store it in the refrigerator at 4^oc until it use. The stock solution was prepared by taking 1 ml of concentrated solution and calculated the dry weight, where it was found that 1 ml contains 0.2 gm of the active ingredient then dissolved this amount in 100 ml of distilled water then prepared series of concentrations required to study : 500,1000,1500 and 2000 ppm.

Effect of *Tagetes erecta* extract in fungus *Paecilomyces lilacinus*

The poisons medium was used to detect the toxic effect of al-Jaafari plant extract in the daily growth rate of *P. lilacinus*, its spore forming and viability, The potato dextrose agar PDA was prepared and distributed in glass flasks (250 ml medium of culture / flask) and sterilized with an autoclave at a temperature of 121^oc and a pressure of 15 kg/cm² for a quarter of an hour then left it to cool after this time treated with aqueous and alcoholic extracts to get the following concentrations (500, 1000, 1500, 2000) ppm, then poured into 9 cm petri dishes and left to harden, then inoculate the center of each dish contain PDA medium with a 1.5 cm diameter tablet of pure fungus at the age of one week, the dishes were incubated at (25 ± 2)^oc for 5 days, then it was calculated the daily growth rate of fungus according to the following equation:

$$\text{The daily growth rate of the fungus} = \frac{D_2 - D_1}{2(T_2 - T_1)}$$

Where, D: means represents the colony diameter, and T: represents time. The average number of spores was calculated on a 1.5 cm diameter disc by selecting

three of 1.5 cm tablets from the fungus treated with the extracts and three tablets non-treated with the extracts for comparison at a distance of 2 cm from the dish. Place each tablet in 50 ml distilled water in, a clean, sterilized, glass flask, moved on the magnetic mixer machine for three minutes, the number of spores was calculated in 1 ml of the bovine suspension using the Haemocytometer (Lomer, 1997).

The toxic effect of the plant extract on the growth, viability and spores forming of the second generation of fungus *P. lilacinus* was investigated by taking 1.5 cm diameter tablets from the first generation of fungus growth on media PDA and cultivating them on pure, unsaturated PDA media. The new dishes were incubated at $25 \pm 2^\circ\text{C}$ for one week, after this was investigate the daily growth rate of the fungus by calculating the number of colonies it has formed.

Results and Discussion

Effect of Al- Jaafari plant extracts on the daily growth rate of fungus *P. lilacinus*

The results that was obtained to study the effect of aqueous and alcohol extracts of Al-Jaafari plant in the daily growth rate of *P. lilacinus* table 1 showed that the extracts with both aqueous and alcohol had a negative effect on the daily growth rate of the fungus and significantly different from the comparison treatment, but it did not stop the growth of fungus even at the highest concentrations that be used, It was noticed through the results that the alcohol extract significantly superior than the aqueous extract in inhibiting the daily growth rate of *P. lilacinus*. The daily growth rate of the fungus was 0.62 cm when treated with the alcohol extract while 0.67 cm when treated with aqueous extract, results as well the effect of the extracts is proportional to the concentration that be used, The effect increases with increasing concentration with no interconnected between them. The results also showed that the daily growth rate of *P. lilacinus* in the case of the aqueous extract at 500

ppm was 0.77 cm compared to the control treatment which was 1 cm. The average daily growth rate of fungi gradually decreased with a concentration, it reach 0.42 cm at the concentration of 2000 ppm during the same time period, In the case of the alcohol extract, the daily growth rate of the fungus was 0.68 cm when treated with a concentration of 500 ppm. The rate was also gradually decreasing with an increase of concentration to 0.37 cm at the concentration of 2000 ppm during the same period.

The effect of the extract with its aqueous and alcoholic on the daily growth rate of *P. lilacinus* may be attributed to the fact that the extract contains compounds with antifungal effect, amount of these substances with the inhibitory effect increased with increasing concentration used. One study indicated that the Al-Jaafari plant contain antifungal compounds, bacteria, insects and nematodes. One of the most important compounds to be observed was Thiophenes (Omer *et al.*, 2015). Another study indicated that the antifungal effect in the al-Jaafari plant is due to the presence of several antimicrobial compounds, the most important of which are derivatives of the compound Monoterpenic, which is a component of essential oils in the leaves and other derived from the stem extract, including three compounds are Piperitone, Dihydrotagetone, α -Terpineol (Donum,1983). Another study observed that the use of 20% concentration of alcoholic extract of the Al- Jaafari plant inhibited the growth of fungi, *Curvularia lunata*, *Drechslera oryzae* and *Fusarium moniliforme* in the rate of growth, 100% (Chowdhury *et al.*, 2015) The growth of the fungus *Colletotrichum gloeosporoides* has been inhibited by 100% and the *Pestalotiopsis guepinii* fungus by 74% at 10% concentration (Shamsie *et al.*, 2014) in a similar study, (Avila-Sosa *et al.*, 2011) indicated that the extract of *T. lucida* inhibited the growth of fungi *Candida albicans*, *Colletotrichum lindemuthianum*, *Saccharomyces cerevisiae*, *Mucor circinelloides* and *Sporothrix schenckii* and *Cespedes*

Table 1: Effect of Al- Jaafari plant extract on the daily growth rate of fungus *Paecilomyces lilacinus*.

Type of extract	Concentrations(ppm)					Avg.
	500	1000	1500	2000	Control	
Aqueous	0.77	0.65	0.53	0.42	1	0.67
Alcohol	0.68	0.57	0.50	0.37	1	0.62
Avg.	0.73	0.61	0.52	0.39	1	-
		Type of extract	Concentrations			Interference NS
L.S.D	0.03		0.04			

Ns: means there is no interference.

et al., (Cespedes *et al.*, 2006) also noted that Dimethoxy compounds were have the main role in inhibiting the growth of the fungus *Rhizoctonia solani* and *Trichophyton mentagrophytes*. It is clear from the results that the extract of the plant Al- Jaafari inhibit the growth of the fungus but does not stop the growth of this, may be attributed to the ability of the fungus to tolerate high concentrations of chemical compounds inhibiting growth and this enhances its use with pesticides or antimicrobials in the fight against insects have been many studies that the fungus

Table 2: Effect of Al- Jaaffari extract in viability of spores of fungus *Paecilomyces lilacinus*.

Number of fungus colony						Concentration ppm
Second generation			First generation			
Avg.	Alcohol extract	Aqueous extract	Avg.	Alcohol extract	Aqueous extract	
45.5	46	45	40	40	40	Control
	44	45	30	28	32	500
44.5	43	43	25	23	27	1000
43	43	41	20.5	18	23	1500
42	42	41	16	14	18	2000
-	43.6	43	-	24.6	28	Avg.
Interference	concentration	Extract	Interference	concentration	Extract	LSD0.05
4.34	3.07	1.94	4.46	3.15	1.99	

P. lilacinus is a fungus that has the ability to withstand high concentrations of certain pesticides and heavy metals in addition to its tolerance to high temperatures, acidity and osmotic potential (Nguyen *et al.*, 2016; Rhee; Gad, 2016; Carranza *et al.*, 2014) In anti - insect control many studies have indicated that *P. lilacinus* fungus characterized by its ability to withstand high concentrations of some pesticides and heavy metals.

Effect off Al- Jaafari extract(*T. erecta*) in the viability of the spores of the fungus *P. lilacinus*

The results to study of the effect of *T. erecta* extract on the viability spores of *P. lilacinus* showed in the table 2 that the plant extract with both aqueous and alcoholic had significant effect on spore viability and a significant difference from the control treatment, this was observed by calculating the number of colonies that formed in 1 ml of spore. When treated with aqueous extract, the average number of colonies formed by fungus was 28, while 24.6 colonies when treated with alcoholic extract with a significant difference between the effect of the two extracts. The effect increased with increasing concentration, when 500 ppm of aqueous extract was used, the number of fungus colonies was 32, the number of colonies gradually decreased at 18 at concentration 2000 ppm after the same period, when the concentration of 500 ppm of alcoholic extract was used, it was noticed that the number of colonies was 28 colonies and this number gradually decreased by increasing the concentration of the extract and for the same period of time until it reached 14 colonies at concentration 2000 ppm and had the lowest growth rate of fungi colonies (the highest effect) in both extracts the average effect was 16, while the concentration 500 ppm had the highest growth rate of fungi colonies (the lowest effect) in both extracts the average effect was 30. The results also showed that the plant extract of both aqueous and alcohol had no effect on the viability of the second generation

spores of the fungus even when the highest concentration of both extracts was used.

It is clear from these results that the extract of aqueous and alcohol has inhibited the viability of the fungus spores under study and this inhibition may be due to the presence of inhibitors, including the essential oils in the tissues of this plant and compounds Thiophenes, which is also characterized by its anti-fungal and other pathogens, has indicated that many studies one of them by (Sundari *et al.*, 2015) in this study to compare the effect of alcoholic extract of *T. patula* and *Datura stramonium* on the growth of the fungus *Macrophomina phaseolina*, where the concentration of 500 ppm has achieved an inhibition rate of 52.19% in the case of *Tagetes* and 50.33% in the case of *Datura*, which indicates that the plant extract can be used in low concentrations to be tolerated by fungus and is more effective against other pathogens, as (Mares *et al.*, 2004) noted that the effect of *Tagetes* spp. on fungi and inhibition of growth caused by the extract changes at the cellular level in general and the plasma membrane in particular. In a similar study (Chaparro *et al.*, 2011) indicated that *T. harzianum* and *T. asperelloides* were able to tolerate the recommended dose for field uses of Captan and also indicated that the activity of the fungus gradually improved over time and that the pesticide at its recommended dose would not affect the efficiency of *T. harzianum* in parasitism on *Fusarium*, they pointed out that the tolerance of this fungus to the recommended pesticide dose is due to physiological molecular changes at the level of DNA occur after the fungus exposure to the pesticide and that these genetic changes reduce the fungus sensitivity to chemical pesticides and therefore does not affect it as a biological control agent it can be used with chemical pesticides or plant extracts that have anti-pathogenic effect.

Effect of *T. erecta* extract on spore forming of

Table 3: Effect of Al- Jaafari extract in spore forming of fungus.

% For inhibition		Concentration ppm
Alcohol extract	Aqueous extract	
-	-	Control
14.3a	11.4a	500
28.5b	27.0b	1000
42.8c	40.0c	1500
57.0d	55.7d	2000

fungus *P. lilacinus*.

The results of the study of the effect of *T. erecta* extract on *P. lilacinus* showed in table 3 that the plant extract, both aqueous and alcoholic, had a negative effect on spore forming of fungi and significantly differentiated with the comparison treatment, but the process of spores did not stop even higher concentration. The results indicated that the effect of aqueous and alcoholic extracts of the Al- Jaafari plant on the spore forming fungi under study is proportional to the concentration was used, the percentage of inhibition of spore forming process is directly proportional to the concentration. Increasing the concentration increases the percentage of inhibition where it is noted that the concentration of 500 ppm has been recorded Inhibition legate 11.4% and 14.3% for each of the aqueous extracts and alcohol respectively, and this percentage increased gradually in both extracts increased focus even reached 55.7% of aqueous extracts and 57% to extract alcohol, also notes the existence of a significant difference between all the concentrations used

Since *T. erecta* extract of both aqueous and alcoholic species has affected the daily growth rate of the fungus table 1 naturally it affects the spore forming, process of the fungus and this effect is increased by increasing the concentration of plant extract with both aqueous and alcohol.. One study indicated that one of the essential compounds responsible for the anti-*Tagetes patula* against *Penicillium digitatum* and *Botrytis cinerea* are essential oils and also indicated that the fungal hypha has shown signs of necrotic necrosis after 24 hours of treatment, Piperitenone and Piperitone are chemical compounds found within the components of these oils that have had a significant effect on these cellular changes (Romagnoli *et al.*, 2005; Deepak *et al.*, 2007) have indicated that the extract of the leaves of the Al-Jaafari (*T. erecta*) has inhibited the forming of conidiospore for *Sclerospora graminicola*,. (Waghmare *et al.*, 2011) noted in another similar study that the leaf extract of *Tagetes erecta* with its aqueous and alcoholic qualities inhibited the growth of *Botrytis cinerea* and spore forming when used at 50% concentration. The alcoholic extract was more efficient than the aqueous extract at 25%

concentration. Another study indicated that the use of plant extract of *Tagetes patula* at a concentration of 20% against the fungus *Botrytis cinerea* inhibited the growth of fungal hypha of the fungus by 88.5% and inhibited the process of spores forming by 100% (Sesan *et al.*, 2015).

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