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# Screening of *Fusarium* isolates pathogenicity *in vitro* by using the larvae of *Galleria Mellonella* L.

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**Abstract**

Eighty soil samples are collected from cultivated soil in different locations in Basrah. A total of 78 isolates were detected and idintified belonging to *F.chlamydosporum* ,*F.equiseti*,*F.graminearum*,*F.moniliforme*,*F.oxysporum* ,*F.poae*,*F.semitectum* ,*F.sacchari* and *F.solani* . The pathogenicity of all *Fusarium* isolates was evaluated towrds larvae *Galleria mellonella* .The mortality percentage of larvae due to infection *Fusarium* isolates ranged from 13-76.6%.*F.oxysporum*3, *F.oxysporum*21,*F.solani* 12and *F.solani*16 were resulted in the highest *Galleria* mortality 76.6% in the pathogenicity test.The results indicated that the pathogenicity of *Fusarium* isolates against *Galleria* larvae was significantly varied.

**Key words:** Pathogenicity ,*Fusariusm* species,*Galleria mellonella.*

## 1.Introdution

Entomopathogenic fungi are widely distributed throughout the fungal kingdom. Some insect-pathogenic fungi have ,restricted host ranges, while others have a wide host range, with individual isolates being more specific [1]. Biological control is an environmentally friendly alternative that involves the use of natural enemies and pathogens to control pests [2].Recently, a large number of insect pathogenic microorganisms referred to as entomopathogens have been identified as possible biological control agents for insects [3].Among these agents,fungal entomopathogens had been reported to have great potentials for insects control [4].*Metarhizium anisopliae*, *Fusarium* sp., and *Beauveria bassiana* can infect different insect species in screen house or field conditions [5;6;7].The genus *Fusarium* comprises a large group of species of filamentous fungi widely distributed in soil usually in association with plants. Most species are saprotrophic and relatively abundant members of the soil microbiota [8]. Many *Fusarium* species are well-known as pathogens of plants, insects, and humans, although the systematic description of this genus is unresolved *Fusarium* is unique because some isolates of the common phytopathogenic species (e.g.those associated with root rot diseases) also can infect subterranean insects [9].More than 13 *Fusarium* species are pathogenic to insects, and the genus has a host range that includes Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera [10;11]. *Fusarium* includes various species/strains that are able to produce potent secondary metabolites, such as trichothecenes [12;13],fumonisins [14] and beauvericin[15;16;17].The latter is a

widespread metabolite among entomopathogenic fungi, such as *Beauveria bassiana* and *Paecilomyces fumosoroseus*[18;19].Insects are often used as reliable organisms for screening the potential pathogenicity and toxicity of fungi[20]. Because of the long-standing interest of insects both as bioassay organisms and as control agents,several studies have been conducted that focus on the isolation and screening of entomopathogenic fungi which may be effective in controlling the pest insects [21;22].Tiago *et al*.[23] have studied the differential pathogenicity of *Metarhizium anisopliae* on the sugarcan root spittebug *Mahanarva fimbriolata*.Sinha [24] compared the toxicity of several species of *Alternaria , Mucor , Hormodendrum* and *Nigrospora* on adults and larvae of *Tribolium* species .Sankar *et al*.[25] had tested the pathogenicity the effect of three fungal pathogens *Metarhizium anisoplia* , *Beauveria bassiana* and *Trichoderma viride* on greater wax moth *Galleria mellonella* larvae under laboratory condition.The aim of this study was to work out an easy bioassay for screening pathogenicity of the isolates of several *Fusarium* species towards *Galleria mellonell* .

## 2.Materials and Methods

### 2.1.A study area and samples collection

Eight sites were selected for collecting samples of soil during the period of March 2007 and March 2009. Ten collections were carried out from each site. These sites represented fields of Abo Al-Khasib ,Al-Qarma,Al-Junaena Al- Zubair, Al-Dair, Brathia, Borjissia and Shatt Al – Arab districts. Fields of Abo Al- Khasib,Al-karma ,Brathia and Shatt Al-Arab were dominated by date palm trees, while the fields of Al-Zubair,Al-Junaena and Borjissia are featured by a desert land and sandy soil with vegetable cultivation , fields of Al-Dair were under Barley and wheat cultivation. Eighty Soil samples (about 500g each) were collected during this study from the depth of 5 – 15 cm with trowel, placed in plastic bag and brought back to the laboratory. Samples were processed within two days of collection .

### 2.2.Isolation and Identification of fungi

Two isolation methods were applied, the direct plating methods [26] and dilution plate method[27]. Two types of media were used for the isolation of *Fusarium* species, a selective medium of Shimazu and sato [28] [Bactopeptone 3 g, $CuCl_2$ 0.2g , crystal violet 2 mg , agar 15g and 1,000 ml distilled water]the medium was adjusted to pH=10,and potato dextrose agar(PDA).Bacterial growth was controlled by the addition of chloramphenicol 100µg/ml.The plates were incubated at 25±1 $C^o$ and inspected for the growth of *Fusarium* species. Fungal pure culture was made for each isolate and identified with the aid of several taxonomic reference [29;30;31;32;33;34].The percentage of occurrence was determined by (number of fungal appeared in samples/ total number of samples of region).The Percentage of isolation from each site(number of isolates per site/ Total number of isolates from the site).

### 2.3.Rearing of *Galleria mellonella*

The greater wax moth *G.mellonella* (Lpidoptera: Pyralidea) was obtained from the laboratory of plant pathology, University of Alicante, Spain. The greater wax moth was reared in constant darkness following the method of Berbegal [35].

**2.4.Pathogenicity test for *Fusarium* isolates to *G.mellonella***

The pathogencity test was performed according to Ali-Shtayeh *et al.*[36]. The tested fungal isolates were grown on PDA plates for 10 days. Sterile water 5ml. was powered on each plate containing fungal colony to obtain spore suspension. Fourth instar larvae of *G.mellonella* were immersed into the spore suspension for few seconds and then transferred into 9 cm. Petri dish with moistened filter paper .Petri dishes were sealed with parafilm to maintain the humidity and then incubated in darkness at 25±1 $C^{o}$ . Infected larvae were inspected and counted daily .Mortality percentage caused by each fungal isolate was calculated after 3,6,9 and 12 days of infection .Re-isolation and identification of the fungus growing on the dead larvae were performed to evaluate whether the fungus was the same as inoculated.

**2.5.Statistical analysis**

Data were analyzed using ANOVA analysis($p<0.05$), the completely randomized design was used in pathogenicity test, original data for the statistical analysis were transformed into ARCSINE. All treatments were replicated three times,ten larvae for each treatment.

**3.Results**

A total of eighty samples was screened for the presence of *Fusarium* species, out of which 58 samples contained 78 isolates .These isolates represent nine species, *Fusarium chlamydosporum,F.equideti ,F.graminearum,F.moniliforme,F.oxysporu m ,F.poae,F.semitectum,F.sacchari* and *F.solani.*Recovery of isolates in each site was: in Al-Zubair and Barjissia with a percentage of occurrence 90%,whereas was 80% in Abo-Al khasib and shatt Al-arab,70% was in Al-Qarma,and 60% was in Al-Junaena and Brathia,the lowest occurrence of *Fusarium* was shown in Al-Dair region with a percentage occurrence of 50%(Fig.1).

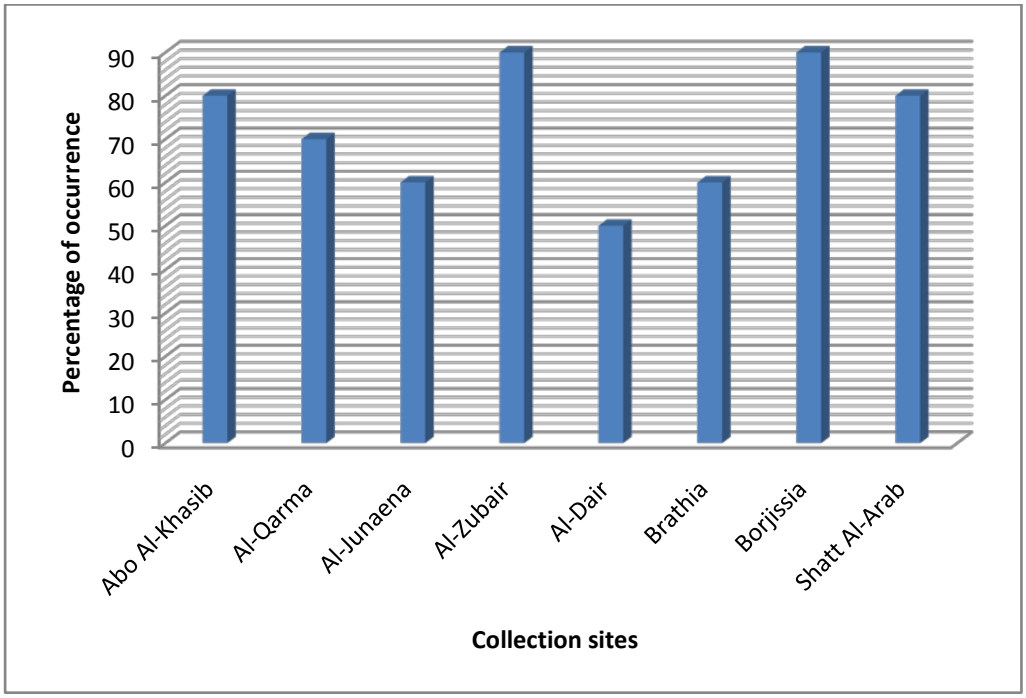


**Fig.1:Occurrence percentage of the genus *Fusarium* in eight sites from soil according to collection sites in Basrah city.**

Among the isolated species *F.oxysporum*(27 of the total isolates) and *F.solani*(24 isolates of 78)were the most abundant in all sites,while *F.moniliforme*

and *F.semitectum*(7isolates for each of 78). *F.poae and F.equiseti* are restricted to sites Al-Qarma and Al-Junaena respectively one isolate for each(Table,1).

**Table(1) Distribution and occurrence percentages of *Fusarium* species in soil by geographical location and vegetation cover.**

| Sampling site | Vegetation cover | Fungal species | No.of fungal isolattes | Isolation percentage |
|---|---|---|---|---|
| Abo-Al-Khasib | Date palm | *Fusarium solani* | 3 | 27.27 |
| | | *Fusarium oxysporum* | 3 | 27.27 |
| | | *Fusarium chlamydosporum* | 1 | 9.09 |
| | | *Fusarium graminearum* | 2 | 18.18 |
| | | *Fusarium moniliforme* | 1 | 9.09 |
| | | *Fusarium sacchari* | 2 | 18.18 |
| Al-Qarma | Date palm | *Fusarium solani* | 3 | 23.07 |
| | | *Fusarium oxysporum* | 4 | 30.76 |
| | | *Fusarium chlamydosporum* | 1 | 7.69 |
| | | *Fusarium semitectum* | 2 | 15.38 |
| | | *Fusarium moniliforme* | 2 | 15.38 |
| | | *Fusarium poae* | 1 | 7.69 |
| Al-Junaena | Vegetable | *Fusarium solani* | 3 | 42.85 |
| | | *Fusarium oxysporum* | 2 | 28.57 |
| | | *Fusarium semitectum* | 1 | 14.28 |
| | | *Fusarium equiseti* | 1 | 14.28 |
| Al-Zubair | Vegetable | *Fusarium solani* | 3 | 37.5 |
| | | *Fusarium oxysporum* | 4 | 50 |
| | | *Fusarium semitectum* | 1 | 12.5 |
| Al-Dair | Barly and Wheat | *Fusarium solani* | 3 | 33.33 |
| | | *Fusarium oxysporum* | 2 | 22.22 |
| | | *Fusarium graminearum* | 3 | 33.33 |
| | | *Fusarium semitectum* | 1 | 11.11 |
| Brathia | Date palm | *Fusarium solani* | 3 | 30 |
| | | *Fusarium oxysporum* | 4 | 40 |
| | | *Fusarium semitectum* | 1 | 10 |
| | | *Fusarium moniliforme* | 2 | 20 |
| Borjissia | Vegetable | *Fusarium solani* | 3 | 37.5 |
| | | *Fusarium oxysporum* | 4 | 50 |
| | | *Fusarium chlamydosporum* | 1 | 12.5 |
| Shatt Al-arab | Date palm | *Fusarium solani* | 3 | 27.27 |
| | | *Fusarium oxysporum Fusarium* | 4 | 36.36 |
| | | *chlamydosporum* | 1 | 9.09 |
| | | *Fusarium semitectum* | 1 | 9.09 |
| | | *Fusarium moniliforme* | 2 | 18.18 |

All the isolates in the present study were tested for their pathogenicity against larvae of *Galleria mellonella*.Data in table (2) showed different degrees of pathogenicity against *G.mellonella* larvae. Highest mortality rates of *G.mellonella* larvae were obtained by isolates of *F.oxysporum* and *F.solani* ranged from 10- 76% and 13-76% respectively after 12 days(Fig.2,3).*G.mellonella* larvae were strongly affected by *F.oxysporum*3,*F.oxysporum*21,*F.solani*12 and *F.solani* 16 which caused 76% mortality after 12 days .These mortality rates were significantly different from other isolates. These indicated the existence of differences in the mortality rates between the isolates. After 3,6 and 9 days of inoculation mortality rates of *F.oxysporum* isolates ranged between 0-6%,0-16% and 10-56% respectively, while mortality rates of *F.solani* isolates for the same periods

ranged between 0-6%,0-16% and 3-43%, respectively.

*F.moniliforme* and *F.semitectum* isolates exhibited a medium virulent against *G.mellonella* larvae which caused 56% and 46% ,respectively, after 12 days of inocubation.Mortality rates of other isolates of *F.chlamydosporum,F,equiseti ,F.graminearum,F.poae* and*F.sacchari* ranged between 13-26% after 12 days of inocubation,with significantly differences between them(Table,2)

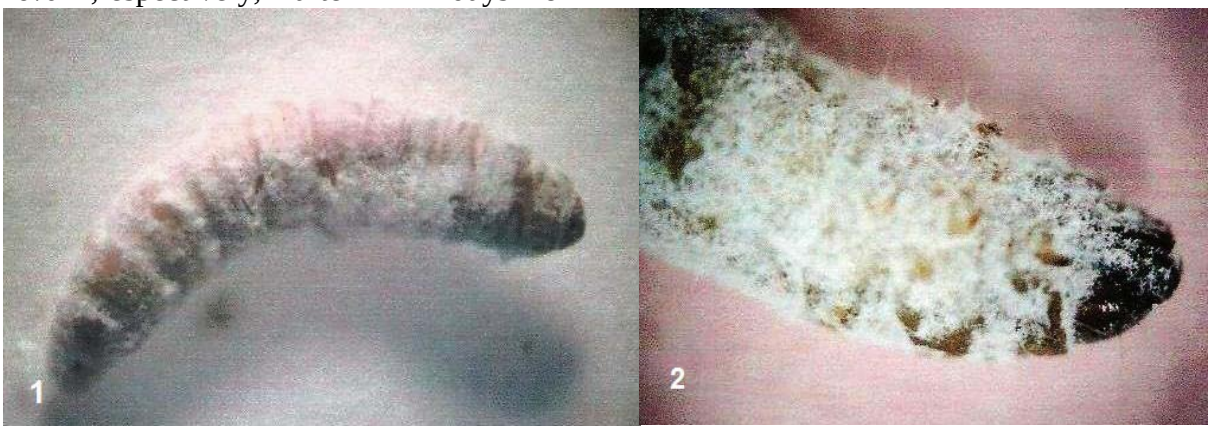


**Fig.1,2: Infected larvae of *Galleria mellonella* with:1(x6.4), *Fusarium oxysporum* and2(x16), *F.solani***

**Table (2) Pathogenicity test of *Fusarium* isolates on *Galleria mellonella* larvae**

| Fungal isolates | Mortality % | | | |
|---|---|---|---|---|
| | 3 Days | 6Days | 9Days | 12Days |
| *Fusarium chlamydosporum1* | 0 | 6 | 10 | 13 |
| *F. chlamydosporum2* | 0 | 3 | 6 | 10 |
| *F. chlamydosporum3* | 3 | 10 | 13 | 20 |
| *F. chlamydosporum4* | 0 | 3 | 3 | 13 |
| *F.eqiseti* | 0 | 0 | 6 | 16 |
| *F.graminearum1* | 0 | 0 | 3 | 13 |
| *F.graminearum2* | 0 | 3 | 6 | 16 |
| *F.graminearum3* | 0 | 0 | 6 | 20 |
| *F.graminearum4* | 0 | 6 | 6 | 20 |
| *F.graminearum5* | 3 | 10 | 10 | 20 |
| *F.moniliforme1* | 0 | 0 | 3 | 13 |
| *F.moniliforme2* | 0 | 3 | 6 | 16 |
| *F.moniliforme3* | 0 | 3 | 36 | 56 |
| *F.moniliforme4* | 0 | 0 | 3 | 13 |
| *F.moniliforme5* | 0 | 6 | 10 | 20 |
| *F.moniliforme6* | 0 | 6 | 6 | 20 |
| *F.moniliforme7* | 0 | 0 | 3 | 16 |
| *F.oxysporum1* | 0 | 3 | 6 | 20 |
| *F.oxysporum2* | 6 | 16 | 56 | 73 |
| *F.oxysporum3* | 3 | 13 | 43 | 76 |
| *F.oxysporum4* | 0 | 3 | 10 | 43 |
| *F.oxysporum5* | 3 | 10 | 20 | 36 |
| *F.oxysporum6* | 6 | 13 | 26 | 40 |
| *F.oxysporum7* | 0 | 3 | 10 | 20 |
| *F.oxysporum8* | 0 | 0 | 6 | 20 |
| *F.oxysporum9* | 0 | 0 | 6 | 16 |
| *F.oxysporum10* | 0 | 0 | 3 | 13 |
| *F.oxysporum11* | 0 | 0 | 6 | 20 |
| *F.oxysporum12* | 0 | 3 | 6 | 23 |
| *F.oxysporum13* | 0 | 0 | 6 | 20 |

| | | | | |
|---|---|---|---|---|
| *F.oxysporum14* | 0 | 6 | 10 | 26 |
| *F.oxysporum15* | 0 | 0 | 3 | 13 |
| *F.oxysporum16* | 0 | 0 | 3 | 16 |
| *F.oxysporum17* | 3 | 10 | 13 | 36 |
| *F.oxysporum18* | 0 | 3 | 10 | 23 |
| *F.oxysporum19* | 0 | 0 | 6 | 16 |
| *F.oxysporum20* | 0 | 0 | 0 | 10 |
| *F.oxysporum21* | 3 | 13 | 40 | 76 |
| *F.oxysporum22* | 3 | 10 | 33 | 63 |
| *F.oxysporum23* | 0 | 0 | 6 | 13 |
| *F.oxysporum24* | 0 | 3 | 6 | 23 |
| *F.oxysporum25* | 0 | 6 | 13 | 33 |
| *F.oxysporum26* | 3 | 10 | 20 | 46 |
| *F.oxysporum27* | 0 | 3 | 10 | 23 |
| *F.poae* | 0 | 0 | 6 | 13 |
| *F.sacchari1* | 3 | 6 | 10 | 26 |
| *F.sacchari2* | 0 | 0 | 3 | 13 |
| *F.semitectum1* | 0 | 3 | 6 | 13 |
| *F.semitectum2* | 0 | 0 | 3 | 10 |
| *F.semitectum3* | 3 | 10 | 20 | 46 |
| *F.semitectum4* | 0 | 3 | 6 | 16 |
| *F.semitectum5* | 6 | 13 | 36 | 63 |
| *F.semitectum6* | 0 | 0 | 3 | 16 |
| *F.semitectum7* | 3 | 10 | 16 | 36 |
| *F.solani1* | 0 | 0 | 3 | 13 |
| *F.solani2* | 0 | 0 | 0 | 10 |
| *F.solani3* | 0 | 0 | 6 | 20 |
| *F.solani4* | 0 | 3 | 6 | 20 |
| *F.solani5* | 0 | 0 | 6 | 16 |
| *F.solani6* | 3 | 6 | 16 | 30 |
| *F.solani7* | 0 | 0 | 6 | 20 |
| *F.solani8* | 0 | 3 | 10 | 26 |
| *F.solani9* | 0 | 6 | 13 | 30 |
| *F.solani10* | 3 | 10 | 20 | 43 |
| *F.solani11* | 0 | 0 | 6 | 16 |
| *F.solani12* | 6 | 16 | 43 | 76 |
| *F.solani13* | 3 | 10 | 30 | 66 |
| *F.solani14* | 0 | 0 | 6 | 20 |
| *F.solani15* | 0 | 3 | 10 | 13 |
| *F.solani16* | 3 | 13 | 43 | 76 |
| *F.solani17* | 0 | 0 | 3 | 16 |
| *F.solani18* | 0 | 0 | 6 | 13 |
| *F.solani19* | 0 | 3 | 10 | 33 |
| *F.solani20* | 0 | 6 | 16 | 36 |
| *F.solani21* | 3 | 0 | 6 | 23 |
| *F.solani22* | 0 | 0 | 3 | 20 |
| *F.solani23* | 0 | 3 | 10 | 20 |
| *F.solani24* | 0 | 0 | 10 | 16 |
| Control | 0 | 3 | 6 | 13 |

R.L.S.D Fungal isolates= 11.6 R.L.S.D periods=17.8

## 4.Discussion

A total of 78 isolates of *Fusarium* were recovered from cultivated soil in different locations in Basrah, the genus *Fusarium* appeared high percentage occurrence of 72.5% comparable to Ameen [37] who found that the genus *Fusarium* occurs in about 83% of soil samples collected from cultivated soils.The percentage of *Fusarium* would appear to be related to the permanence of the habitat,possibly because permanent habitats support larger, more diverse and stable communities of insects.Ploughing of cultivated soils may also prevent the building-up of high populations of insect pathogens to adverse environmental conditions on the surface of soil or by burying them away from potential hosts[38].

The four *Fusarium* species *F.moniliforme,F.oxysporum,F.semitectum* and *F.solani* recovered in this study have also been reported from agricultural soil in the Palestinian area [36].Both *F.oxysporum* and *F.solani* have also been recovered by Sun and Liu[39] and Sun *et al.* [40] as opportunistic pathogen from natural and field crops soil in China.Several *Fusarium* isolates *F.oxysporum* 3 , *F.oxysporum* 21,*F.solani* 12 and *F.solani* 16 were caused 76% of mortality to the infected larvae after 12 days ,other isolates were recorded between 10%to 66% of percentage mortality after 12 days,however,this is may be the virulence can be independent of host or location derived from [41].The genus *Fusarium* display agreat diversity of life strategies including a variety of types of associations with insects.*Fusarium* species can act as weak to virulent[42] . According to our observations within a given *Fusarium* species isolates can highly differ in their efficacy to specific targets. Such intraspecific variability for entomopathogenic fungi efficacy has been previously reported by other authors. Mortality of thrips ranged from 0 to 76.6% when inoculated with different isolates of *B. bassiana*. Isolates of *M. Anisopliae* caused mortality in mealybug between 0 to 73.3%[39]. Vandenberg [43] found a high variability among eight isolates of *P. fumosoroseus* towards Russian wheat aphid, *Diuraphis noxia* and Ferron *et al*. [44] and Vicenyini *et al*. [45] described high intraspecific variability in pathogenicity in bioassays with isolates of *B. bassiana*, *P. fumosoroseus* and *M. anisopliae*. In contrast, only a relatively weak intraspecific variability for pathogenic activity was observed among 30 isolates of *P. fumosoroseus* towards *B. argentifolii* with nymphal mortality ranging from 14.6 to 94% [46].

This result is in agreement with Loureio et al.[47] who found low confirmed mortality when compared the 27 isolates obtained from the spittlebugs across different regions in Brazil and only two isolates showed mortality between 80 -88%on the sixth day,Tiago et al.[23] tested differential pathogenicity of nine isolates of *Metarhizium anisoplae* on spittlebug *Mahanarva fimbriolata* and they found that,three isolates revealed a high mortality.

In conclusion, the present findings suggest that *G.mellonella* larvae are reliable sensitive test for screening *Fusarium* speceis pathogenicity as entomopathogenic fungi

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