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ORYCTES ELEGANS, A VECTOR OF FUSARIUM PROLIFERATUM CAUSING WILT DISEASE SYMPTOMS OF DATE PALM

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A B S T R A C T

Fusarium proliferatum is an important pathogen worldwide causing devastating diseases in many crucial crops such as date palm, banana, mango, maize, rice, asparagus, onion, and garlic varied between wilt and dieback. Many insects are able to transmit fungi either during feeding process or mechanically via insects' movement or laying egg operation. *F. proliferatum* was isolated from date palm borer *Oryctes elegans* from five locations in the south of Iraq: Al- Namania, Al-souira, AL- Azezia, Al- Madaan, and Al-Dewania. The fungus was isolated from both insects' adults and plant materials from trees that showed wilt symptoms. The results revealed that there was high frequency of fungal isolation from both materials expressing the theory of transmitting *F. proliferatum* by date palm borer. Al- Madaan samples revealed the highest isolation frequency from both borer adults and plant materials with 62.85% and 63.63% respectively; followed by AL- Azezia which showed the highest isolation frequency from insects with 48.33%; while, Al- souira scored the second highest isolation frequency from plant materials with 52.38%.

Keywords: *Oryctes elegans*, *Fusarium proliferatum*, Date palm, Vector, Wilt disease symptoms.

INTRODUCTION

F. proliferatum is a worldwide spread pathogen infecting large number of crops including date palm, banana, mango, maize ,rice, asparagus, onion, and garlic (Jurado *et al.*, 2010). It is one of the most devastating Fusarium species that causes diseases such as rot, wilt, and die-back. It was recorded in Saudi Arabia as the main causative pathogen of wilt and die-back diseases in date palm trees (*Phoenix dactylifera* L.)(Abdalla, 2000). Hameed (2012) reported that *F. proliferatum* causes Inflorescence rot disease of date palm trees leading to a remarkable yield losses in the south of Iraq. This pathogen was also isolated from palm trees in high frequency level in the Canary Islands(Hernández-Hernández *et al.*, 2010). It was isolated from palm trees in Spain as well causing wilt and die-back (Armengol *et al*., 2005). This necrotrophic pathogen produces number of toxins such as fusaric acid, moniliformin, fusaproliferin fumonisins, beauvericin, and bikaverin (Seefelder *et al.*, 2002, Kohut *et al.*, 2010, Stankovic *et al*., 2007). Date palm borer *Oryctes elegans* Prell is an important pest of date palm, oil, and coconut in Southeast Asia, Middle East, North Africa, and some Pacific islands (Ragoussis *et al.*, 2007, Rochat *et al*., 2004, Hallett *et al.*, 1995). The highest effect was recorded on young trees and the fruit-stalk in producing trees (Rochat *et al*., 2004). Both adults and larvae cause damages on trees through their feeding behavior; for example, *Oryctes elegans* adults mining the stalk of fruit bunches feeding on living tissues; while larvae feed on both living and dead tissues (Rochat *et al.*, 2004). Larvae develop in the crown and at the margin of the stem, feeding at the boundary of dead and living tissues (Hallett *et al*., 1995, Rochat *et al*., 2004).

Insects can be an active vector of pathogens disseminating the disease through larger area via their feeding movements(Agrios, 1980). Dutch elm disease that caused by *Ophiostoma ulmi* is an example of the diseases that are transmuted by beetles on trees. The causative fungus of this disease (*O. ulmi*) infects the wood of living elms or dead trees or cut logs; where, bark beetles develop under the elms. *O. ulmi* spores attach to

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the recently emerged adults' of bark beetles, providing inoculums that will be speared to the new location (Purcell and Rodrigo, 2005). *Nematospora coryli* is an important pathogen causing damages to citrus fruits in Cuba which is transmitted by *Nezara viridula* (Mitchell, 2004). *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum*, *Bipolaris oryzae* were isolated from the saliva and stylets of the rice stink bug(Lee *et al*., 1993). Feldman, O'Brien *et al*.(2008)reported that there is an overlap between fungal dissemination and plant tissue with the role of moth insects as a major vector of the causative pathogen.

This study investigated the role of *O. elegans* in transmitting the pathogen *F. proliferatum* on date palm trees which is the causative pathogen of wilt diseases symptoms and the relationship between the presence of date palm borers and *F. proliferatum*.

MATERIALS AND METHODS

Collection of Samples: Samples were collected from five locations which were severely infested by the date palm borers and showed the symptoms of wilt diseases: Al-Namania, Al- souira, AL- Azezia, Al- Madaan, and Al-Dewania. The fungus *F. proliferatum* was isolated from the collected samples which included both plant materials (leaves and shoots) and date palm borers' adults. The insects' adults were collected by using Pheromones and light traps that distributed in number of orchids in the five locations. Borer adults were collected from April to July which is the period of active season.

Fungal Isolation: Three (2 mm) pieces of tissue from each sample were used to get fungal isolates. The pieces were surface sterilized in bleach (1% available chlorine) for 3 minutes, and washed twice in sterile water for 3 minutes. Then, pieces were dried by placing them on sterile paper towel. Subsequently, samples tissue pieces were transferred onto quarter-strength potato dextrose agar (PDA) plates which contain 100 µg streptomycin sulphate and 10 µg tetracycline hydrochloride mL^{-1}. Plates were incubated at ambient temperature and placed under standard white fluorescent light (35098 F18E/33 General Electric, USA) for 24 hours for 5 -7 days. Spore suspension was prepared by adding 3-4 drops of sterile distilled water on the fungal colony that were grown around the plated tissues using sterile flame-sterilized loop. This spore suspension was streaked onto 2% water agar medium by using a flame-sterilized metal loop and plates were incubated under laboratory conditions for 24 h. A single germinated macroconidium was transferred onto full-strength PDA medium plate and incubated at ambient temperature according to Scott and Chakraborty (2010). Borer adults were cut into small pieces (Fig. 2) under sterile conditions in the laminar air flow and surface sterilized using bleach (1% available chlorine) for 3 minutes and washed twice in sterile water for 3 minutes. Then, pieces were dried by placing them on sterile paper towel. They were transferred onto quarter-strength potato dextrose agar (PDA) plates which contain 100 µg streptomycin sulphate and 10 µg tetracycline hydrochloride mL^{-1}. Spore suspension was prepared by adding 3-4 drops of sterile distilled water on the fungal colony that were grown around the plated tissues using sterile flame-sterilized loop. This spore suspension was streaked onto 2% water agar medium by using a flame-sterilized metal loop and plates were incubated under laboratory conditions for 24 h. A single germinated macroconidium was transferred onto full-strength PDA medium plate and incubated at ambient temperature.

Isolates identification: Obtained isolates (Fig. 2) were identified using microscopic characteristics that classified the fungal pathogen *F. proliferatum*. Fungal isolates were identified according to Booth and Taylor (1976) and (Nicholson, 2007).

Pathogenicity Test: Fungal isolates were grown at 25°C onto full PDA medium for ten days before preparing spore suspension for the inoculation. The inoculums were prepared by adding 10 ml of sterile distilled water to the 7 days old fungal petri dishes and scrapped by metal scraper. Then the harvested spore solution was poured into a 50ml Falcon tube after filtering the solution through sterile Miracloth. The spore concentration was determined using haemocytometer and concentration adjusted to 1×10^6conidia/ml. Two treatments were done via preparing three seedlings for each treatment each seedling were planted in one pot; then, seedlings from one treatment was inoculated via spraying spore suspension and covered by plastic bag, other seedlings were injected by 2ml of spore suspension into the crown area using a hypodermic needle and syringe and covered with plastic bag (Hameed, 2012). The control was prepared by spraying sterile distilled water for one treatment and injecting SDW for the second one. The pots were kept in the green house at 24 ±3 °C and symptoms were observed after six weeks. The appearance of necrotic lesions was indicator of infection, the pathogen then re-isolated from infected sites to confirm the pathogenicity test.

Figure 1. Light traps that were used to collect borers' adults from date palm trees *Phoenix dactylifera* L.

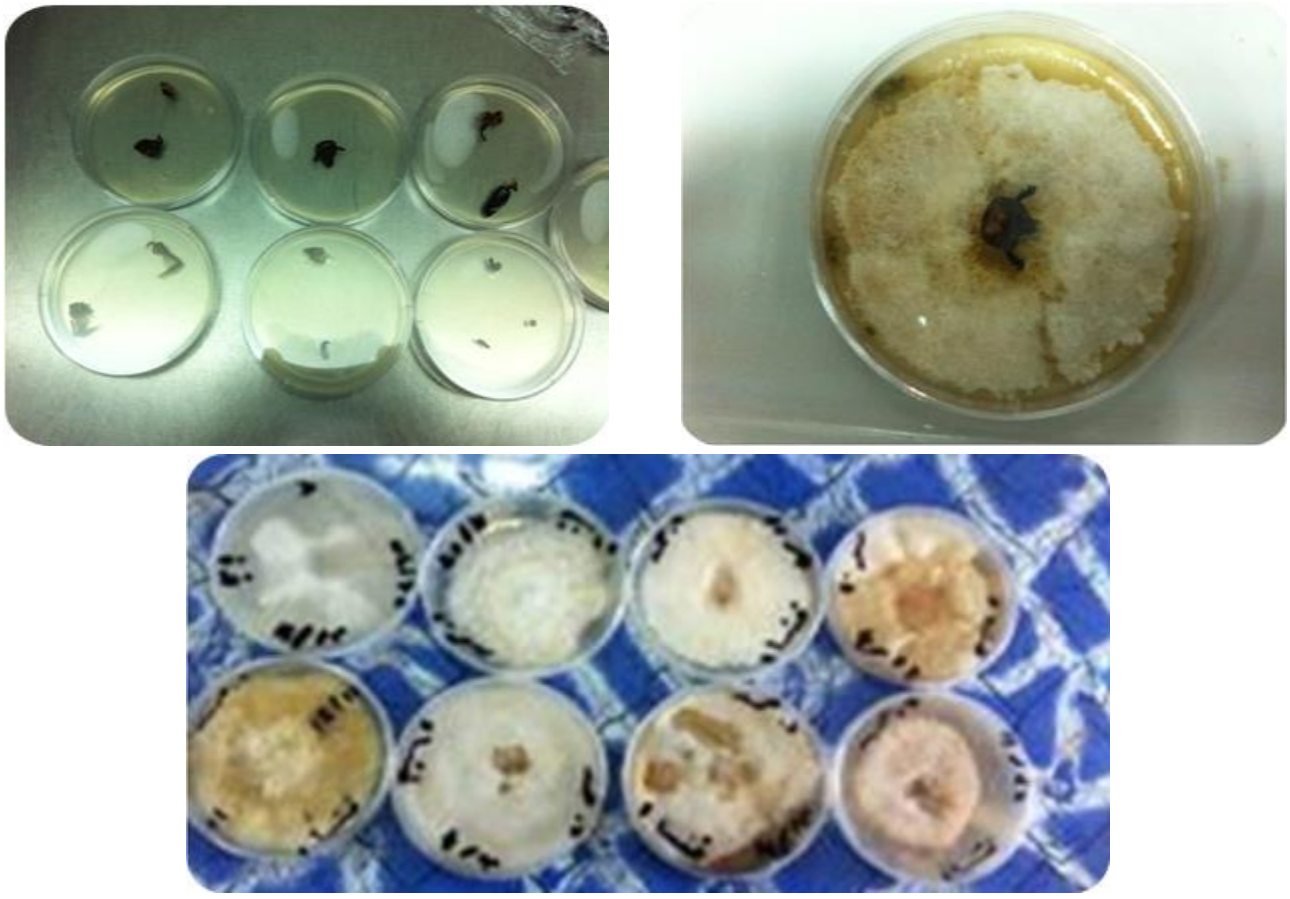

Figure 2. *F. proliferatum* growing in culture isolated from borer adults.

RESULTS

The isolation result of *F. proliferatum* from *O. elegans* showed that the fungus isolated from all tested locations with different percentages of isolation frequency (Table 1). The highest isolation frequency of *F. proliferatum* was recorded at Al- Madaan location with 62.85% followed by AL- Azezia with 48.33%; while, Al-Dewania revealed the lowest percentage of isolation frequency with 25.45%.

The results of isolation *F. proliferatum* from date palm trees revealed that the fungus was isolated from all tested locations with different percentages of isolation frequency (Table 2). The results of isolation from date palm trees confirmed the obtained results from borer adults' isolation, Al- Madaan location showed the highest percentage of *F. proliferatum* isolation frequency with 63.63%; while, Namania recorded the lowest isolation frequency with31.81%.

Table1. The frequency of fungal isolates from borer adults that isolated from the five locations.

Location	Number of adults used for isolation	Number of isolates	Isolation frequency %
Al- Namania	50	20	40
Al- souira	49	17	34.69
AL- Azezia	60	29	48.33
Al- Madaan	35	22	62.85
Al-Dewania	55	14	25.45

Table2. The incidence frequency of *F. proliferatum* isolates from infected date palm trees.

Location	Number of the examined trees	Number of isolates by examined trees	Isolation frequency %
Al- Namania	22	7	31.81
Al- souira	21	11	52.38
AL- Azezia	24	12	50
Al- Madaan	22	14	63.63
Al-Dewania	25	8	32

Figure 3 (a, b, and c). Damages caused by *O. elegans* and the wilt symptoms caused by *F. proliferatum* on date palm.

Pathogenicity test on seedlings revealed symptoms 6 weeks after inoculation with 0.3 cm lesion around injection area; then, lesion increased reaching over 2cm after 8 weeks. After that, seedlings start wilting 12 weeks later and collapsed in comparison with the control treatments that were healthy. The main propose of this treatment is to examine the isolated pathogen ability to cause wilt symptoms and determine it as the main cause of wilt in date palm trees in the examined plant materials.

DISCUSSION

There was a clear relationship between the presence of date palm borer and the incidence of the pathogen *F. proliferatum.* High number of the infested trees by *O. elegans* was shown the symptoms of wilt. Isolating the fungus from borer adults with this high frequency suggested that date palm borer adults were transmitted the pathogen *F. proliferatum* which was the causative of wilt in many countries and regions such as Saudi Arabia and Pakistan. Beetles can transmit fungi as mentioned by Purcell and Rodrigo (2005) which reported the example of Dutch elm disease that is transmitting by the beetles mechanically especially during lying egg stage. Along with the direct damage of the presence of date palm borers as manifested by reducing crop production, transmitting the fungi such as *F. proliferatum* can double the harm via killing the tree.

This possible relationship between wilt symptoms and the infested trees by borers might explain the mechanism of the disease movement from area to another and from tree to another covering large distance. *F. proliferatum* was reported in Egypt as a cause of leaf spot on date palm trees(Farrag and Abo-Elyousr, 2011). In Iraq was isolated from the Inflorescence as the main causative pathogen of Inflorescence rot disease on date palm (Hameed, 2012). However, most of the previously

mentioned papers did not report any connection with the mechanism of diseases movement or if there is any connection with the presence of insects.

This paper expresses the isolation of *F. proliferatum* from borer adults as an evidence of transmitting the pathogen, increasing infection via large area. Moreover, the pathogen was isolated from plant materials of date palm trees that showed the symptoms of wilt (Fig. 3).

Further studies should focus on investigating the mechanism of transmitting *F. proliferatum* by date palm borers along with if there is any more fungi that might be transmitted by *O. elegans* or other date palm borers. Therefore, larger research programs should study which part of the insect's adult is responsible of introducing the fungus to other date palm trees; moreover, investigating the role of date palm borers of increasing wilt disease symptoms date palm trees; also, the interaction between environmental conditions, varieties of date palm and pathogens.

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REFERENCES

Abdalla, M. Y., AL-Rokibah A.,Moretti A. and Mule G. 2000. Pathogenicity of Toxigenic Fusarium proliferatum from Date Palm in Saudi Arabia. *Plant Disease,* 84**,** 321-324.

Agrios, G. N. 1980. Insect involvement in the transmission of fungal pathogens. *In K. Maramorosch & K.Harris (eds.), Vectors of plant pathogens.* New York: Academic Press,467p.

Armengol, J., Moretti, A., Perrone, G., Vicent, A., Bengoechea, J. A. and Garcia-Jimenez, J. 2005. Identification, incidence and characterization of Fusarium proliferatum on ornamental palms in Spain. *European Journal of Plant Pathology,* 112**,** 123-131.

Booth, R. H. & Taylor, G. S. 1976. Fusarium diseases of cereals: XI. Growth and saprophytic activity of Fusarium nivale in soil. *Transactions of the British Mycological Society,* 66**,** 77-83.

Farrag, E. S. H. & Abo-Elyousr, K. A. 2011. Occurrence of Some Fungal Diseases on Date Palm Trees in Upper Egypt and its Control. *Plant Pathology Journal,* 10**,** 154-160.

Feldman, T., O'brien, H. an ARNOLD, A. E. 2008. Moths that Vector a Plant Pathogen also Transport Endophytic Fungi and Mycoparasitic Antagonists. *Microbial Ecology,* 56**,** 742-750.

Hallett, R., Perez, A., Gries, G., Gries, R., Pierce, H., JR., Yue, J., Oehlschlager, A. C., Gonzalez, L. & Borden, J. 1995.

Aggregation pheromone of coconut rhinoceros beetle,Oryctes rhinoceros (L.) (coleoptera: Scarabaeidae). *Journal of Chemical Ecology,* 21**,** 1549-1570.

Hameed, M. A. 2012. Inflorescence rot disease of date palm caused by Fusarium proliferatum in Southern Iraq. *African Journal of Biotechnology,* 11**,** 8616-8621.

Hernandez-Hernandez, J., Espino, A., Rodriguez-Rodriguez, J. M., Perez-Sierra, A., Leon, M., Abad-Campos, P. and Armengol, J. 2010. *Survey of diseases caused by Fusarium spp. on palm trees in the Canary Islands*.

Jurado, M., Marin, P., Callejas, C., Moretti, A., Vazquez, C. and Gonzalez-Jaen, M. T. 2010. Genetic variability and Fumonisin production by Fusarium proliferatum. *Food Microbiology,* 27**,** 50-57.

Kohut, G., Olah, B., Adam, A. L., Garcia-Martinez, J. and Hornok, L. 2010. Adenylyl cyclase regulates heavy metal sensitivity, bikaverin production and plant tissue colonization in Fusarium proliferatum. *Journal of Basic Microbiology,* 50**,** 59-71.

Lee, F. N., Tugwell, N. P., Fannah, S. J. and Weidemann, G. J. 1993. Role of Fungi Vectored by Rice Stink Bug (Heteroptera: Pentatomidae) in Discoloration of Rice Kernels. *Journal of Economic Entomology,* 86**,** 549-556.

Mitchell, P. L. 2004. Heteroptera as Vectors of Plant Pathogens. *Neotropical Entomology,* 33**,** 519-545.

Nicholson, P. 2007. The Fusarium Laboratory Manual - by John Leslie. *Plant Pathology,* 56**,** 1037-1037.

Purcell, A. H., ALmeida Rodrigo P.P. 2005. *Insects as Vectors of Disease Agents*.

Ragoussis, V., Giannikopoulos, A., Skoka, E. and Grivas, P. 2007. Efficient Synthesis of (±)-4-Methyloctanoic Acid, Aggregation Pheromone of Rhinoceros Beetles of the Genus Oryctes (Coleoptera: Dynastidae, Scarabaeidae). *Journal of agricultural and food chemistry.,* 55**,** 5050-5052.

Rochat, D., Mahammad Poor, K., Malosse, C., Avand-

Faghih, A., Lettere, M., Beauhaire, J., Morin, J.-P., Pezier, A., Renou, M. and Abdollahi, G. A. 2004. Male Aggregation Pheromone of Date Palm Fruit Stalk Borer Oryctes elegans. *Journal of Chemical Ecology,* 30**,** 387-407.

Scott, J. B. and Chakraborty, S. 2010. Genotypic diversity in Fusarium pseudograminearum populations in Australian wheat fields. *Plant Pathology,* 59**,** 338-347.

Seefelder, W., Gossmann, M. and Humpf, H. U. 2002. Analysis of Fumonisin B1 in Fusarium proliferatum-Infected Asparagus Spears and Garlic Bulbs from Germany by Liquid Chromatography–Electrospray Ionization Mass Spectrometry§. *Journal of Agricultural and Food Chemistry,* 50**,** 2778-2781.

Stankovic, S., Levic, J., Petrovic, T., Logrieco, A. and Moretti, A. 2007. Pathogenicity and mycotoxin production by Fusarium proliferatum isolated from onion and garlic in Serbia. *European Journal of Plant Pathology,* 118**,** 165-172.