Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Diabetic Foot

Lamyaa Sabeh Abdullah *. Asst.Prof.Dr. Ebtesam thamer jeaz mezeel * Asst.Prof .Dr. Ahlam Ali Sakhi
*Al-Qadisiyah University / College of Education/ Department of Biology, Iraq
Corresponding. Author. E-mail: sci.bio.ph.20.14@qu.edu.iq Ebtesam. Jeaz@qu.edu.iq.com

Abstract

The present study has been designed to investigate of some virulence factor of *Aspergillus niger* in diabetic patients in Al-Dewaniyah city from2022 -2023. Samples were collected during the period from 1/3 to 1/9/ and by 200 random samples from the hospital from the reviewers. Sugar was measured for people with type 2 diabetes and for both sexes. It included the diabetes center in the hospital, health centers and private clinics, as the study included collecting samples from multiple regions. The results found that there is a significant difference at the level of probability ($P \leq 0.05$) between the isolates and samples taken from patients suspected of having diabetes to detect Microbial infections that were cultured on Potato Dextrose Agar to isolate fungi. The results showed when they were electrophoresed on the medium of the agarose gel, and the samples were diagnosed using modern methods for accurate detection, which is the PCR technique and the sequencing technique using the fungus gene 18sRNA and it was 194 bp in size. All samples of *Aspergillus niger* isolates, which are proven in the table below, were subjected to the ability of the fungus to produce biofilms, where the

results of electrophoresis for the alpha-1,3 glucan gene on the agarose gel showed 100% positive results for producing biofilms, and the gene size was 313 bp. *Aspergillus niger* has a high capacity. On the adhesion and production of biofilms, where biofilms developed with high production efficiency.

Key Words : Diabetic Foot , Adhesion , Fumonisin, *Aspergillus niger*

Introduction

Diabetes mellitus is a group of endocrine disorders characterized by high blood sugar and insulin deficiency. There are many cases of diabetics, including type 1 and type 2. The first type represents 10-15% of all patients, which occurs due to the destruction or deficiency of beta cells. In the pancreas and it is no longer able to produce insulin in sufficient quantity or stops, and it is during childhood and adolescence, and it is called (T1DM) Type 1 Diabetes Mellitus, a serious disease that shows clear symptoms on the patient, including hunger, weight loss, frequent urination, high glucose, increased ketone acid, and fluid loss (Nordwall et al. 2009). The second type represents 85-95% of diabetic patients. It occurs due to abnormal secretion of insulin in later stages of life, or because the body is unable to effectively use insulin. The two types differ from each other (Chipkin et al., 2011). Insulin is a hormone that regulates blood sugar levels and is secreted by the pancreas (WHO, 2015). One of the statistics indicated that the number of people with diabetes reaches 170 million people at the present time, and it is likely that by 2030 it will double the number, and that people with diabetic foot ulcers reach 15% of the patients that affect the area under the heel of the foot, where they are a

source of to many nail, tissue and bone marrow infections (Preuyah et al 2014). As there are many organisms that cause infections, the most influential are bacteria and fungi, because they possess many or multiple types of virulence factors in addition to their resistance to many antibiotics, in addition to the neuropathy that accompanies the injury, which causes loss of sensation in the patient, which leads to an exacerbation of the infection (Perkins et al.,2010). Diabetic foot is the most common complication of diabetes that is associated with diabetes in older patients, and the risk of foot ulcers and amputation of the extremities increases, the older the age, in addition to the sugar accompanying the patient for a long time, attention must be paid to the diabetic foot because it is a health problem that leads to diabetic neuropathy and peripheral arterial disease and affects the fibers Nervous and sensory for a patient, which leads to a loss of sense of pain, pressure, and temperature, which leads to an exacerbation of the injury, in addition to several causes, including external and internal, as a result of repeated minor injuries, including nails and foot deformities, and external ones such as shoes, burns, collision with solid objects, and as a result of late detection, the condition leads to ulceration and amputation foot or toes (Lebrun et al., 2010). Complex diabetes develops from ulcers, which is the reason for hospitalization of patients, and that high sugar that occurs as a result of a multifactorial disorder that is characterized by a disorder in the metabolism of carbohydrates, fats, and proteins, and constitutes one of the ten main causes that lead to death (Nabil and Saadallah,2003; Game et al. al., 2012). Diabetes is a serious public health problem all over the world, despite the lifestyle and diet, in addition to the genetic factor, which is one of the main determinants of the risk of developing this disease (Wild et al., 2004). Diabetes is accompanied by other secondary

diseases such as retinopathy, kidney disease, diabetic foot, in addition to onychomycosis where the toenails become thick, causing pressure and irritation (Peter et al., 2009). The nails of a diabetic foot patient are damaged and deformed as a result of diabetes, which causes serious complications for the diabetic patient, which has severe effects and damage to the diabetic foot, which may be the first two reasons. occasion (Oppel & karting, 2003). Infection occurs over the age of 35 years, and genetic history plays a role in the infection, and amputation results from lack of follow-up and adherence to the treatment regimen. There are many wrong habits that diabetics should avoid, in addition to daily examination and moisturizing the skin with paints (Mohammed et al.,2015).

Materials and Methods

Collection of samples

Samples were collected during the period from 1/3 to 1/9/ and by 200 random samples from the hospital from the reviewers. Sugar was measured for people with type 2 diabetes and for both sexes. It included the diabetes center in the hospital, health centers and private clinics, as the study included collecting samples from multiple regions. In the patient, and according to the place of infection, it included urine, nails, skin, and a swab from the foot. Samples were collected in different ways, and the common methods of collecting samples were followed, where urine samples were collected by special tubes or ampoules. Circumstances, or if the skin was damaged after scraping, it was also transferred by tubes. Either a foot swab by means of a special SWAP containing a nutritional medium, in addition to making a specific form for each patient that included the patient's pathological record,

and the form was kept in a special record.

PDA

The medium was prepared from the potato extract, where the medium was used to isolate and diagnose fungi, and it is a good medium for the growth of fungi, as it was prepared at home using 200 gm of potatoes that were boiled with distilled water, then filtered by a piece of sterile gauze, 20 gm dextrose medium was added and autoclaved at 121 C for 15 minutes In addition, chloroformincol is added to it after it cools down to prevent bacterial growth.

Primers: The PCR primers were designed in this study using a database by the company (Sientific Resercher. Co. Ltd in Iraq) and according to Table (1).

Table (1) Primers detection of fungi and virulence factor genes

Primer	Sequence (5'-3')		Product Size	Genbank
***Aspergillus niger* 18S rRNA gene**	F	CCTCCCATCCGTGTCTATTGT	192bp	AJ876876.1
	R	GCCGGAACCAAGAGATCCAT		
***Aspergillus niger* fumonisin biosynthetic fum1 gene**	F	TAGTCTTCGTCCGGTCAGGT	622bp	KJ934797.1
	R	GTGTAACTTTGCTGCGCCAA		
***A. niger* protease (pepD) gene**	F	AGTGGACACTGGCATCTTGG	449bp	L19059.1
	R	GTGGACAGTTCAGCAGAGCT		
***A. niger* lipase lipA gene**	F	TTCTGGAAGGCGTGGGAATC	322bp	AB570425.1
	R	ATTCCGGACTTGGCTGACTG		
***A. niger* biofilm alpha-1,3-**	F	GTAGTCTGCCCAACCCTGAC	313bp	AY549567.1

glucan synthase gene	R	GCGATCCTCCCCAGCTTAAT		
A. niger adhesin gene	F	CCTTCACCTTCCTCCTAGCC	357bp	BCMY01000009.1
	R	GCACCTCAGAACACAACTGC		

Results

Isolation and bacteriological diagnosis

Two hundred samples were collected that were isolated from people suffering from diabetes mellitus of both sexes and of different ages from different hospitals and private clinics in Al-Qadisiyah Governorate. According to Table (2), sequentially, and through statistical analysis, it was found that there is a significant difference at the level of probability ($P \leq 0.05$) between the isolates and samples taken from patients suspected of having diabetes to detect Microbial infections that were cultured on Potato Dextrose Agar to isolate fungi.

Table 2: Types of fungi isolated

	N	%
Aspergillus Niger	**8**	**25.0**
Candida Spp	**8**	**25.0**
Aspergillus Flavus	**8**	**25.0**
Penicillium Spp	**6**	**18.8**
Fusarium	**4**	**12.5**
Aspergillus Ochraceus	**4**	**12.5**
Aspergillus Terreus	**1**	**3.1**

Molecular characterization of fungi

After extracting the DNA of the fungal isolates that were isolated and numbered 8 belonging to the *Aspergills niger*, the results showed when they were electrophoresed on the medium of the agarose gel, and the samples were diagnosed using modern methods for accurate detection, which is the PCR technique and the sequencing technique using the fungus gene 18sRNA and it was 194 bp in size, as shown in the Fig (1).

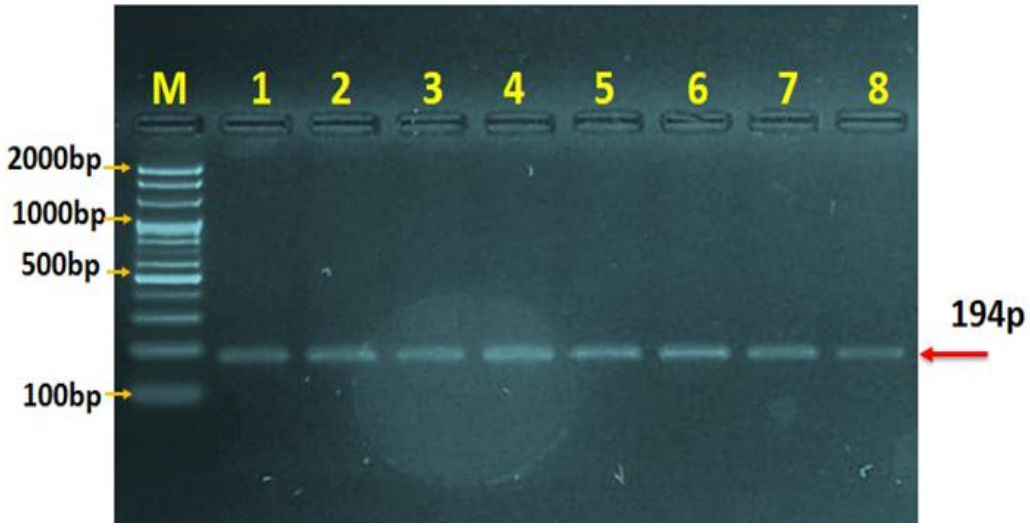

Fig 1: The electrophoresis of the agarose gel, the reaction of polymerase 18 S to detect *Aspergillus niger*

Molecular study of the genes of virulence factors of fungi

Biofilms

All samples of *Aspergillus niger* isolates, which are proven in the table below, were subjected to the ability of the fungus to produce biofilms, where the results of electrophoresis for the alpha-1,3 glucan gene on the agarose gel showed 100% positive results for producing biofilms, and the gene size was 313 bp. *Aspergillus niger* has a high capacity. On the

adhesion and production of biofilms, where biofilms developed with high production efficiency. Fig (2), Table (3).

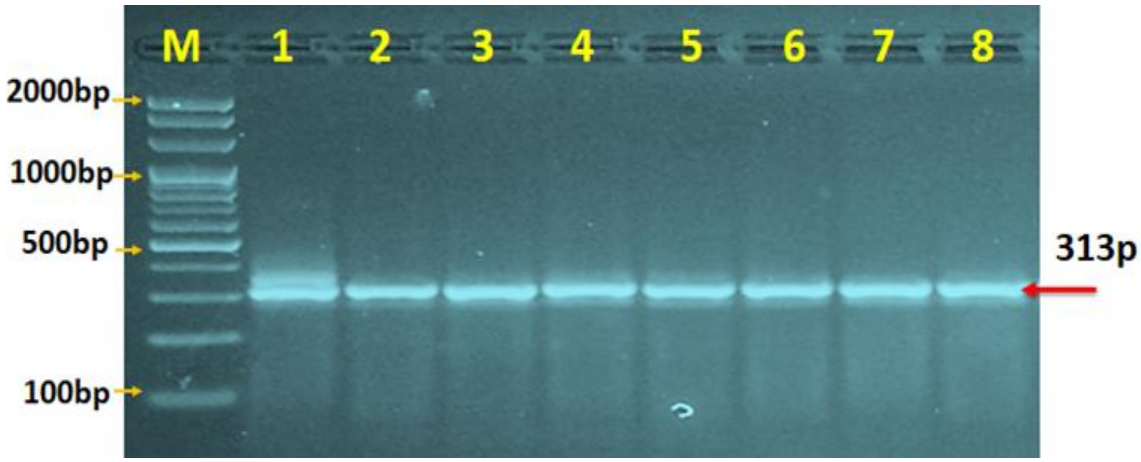

Fig (2): The electrophoresis of agarose gels that showed the polymerase chain reaction product of the alpha-1,3glucan gene from *A. niger* isolates with a size of 313bp.

Table (3) showing the detection of the alpha-1,3glucan gene in *A. niger* isolates

alpha-1,3-glucan synthase gene	Frequency
Results	**N (%)**
Positive, *n* (%)	**8 (100.0%)**
Negative, *n* (%)	**0 (0%)**

Adhesion

The polymerase chain reaction was used to find two molecules of the adhesion gene in *A. niger* isolates (100.0%). The size of the gene was 357 bp as shown in Fig (3), Table (4).

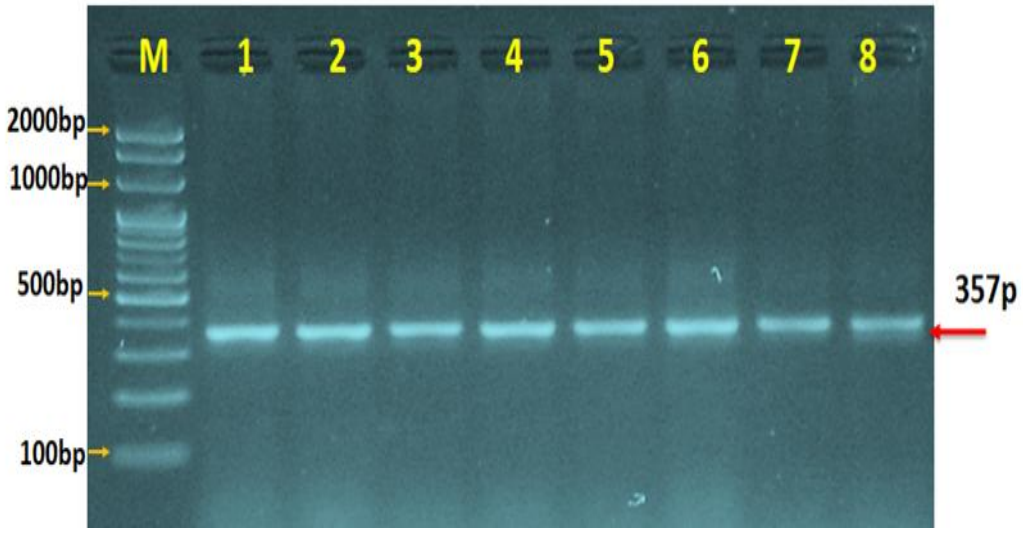

Fig (3): The migration of agarose gel electrophoresis, which showed the analysis of the polymerase chain reaction product of the adhesion gene in *A. niger*, and the size of the gene was 357 bp.

Table (4) shows the detection of the adhesion gene in *Aspergillus niger*

Adhesion gene	**Frequency**
Results	**N (%)**
Positive, *n* (%)	**8 (100.0%)**
Negative, *n* (%)	**0 (0%)**

Fumonisin enzyme

Using polymerase chain reaction to find the molecular gene for the fumonisin enzyme produced by 8 isolates of the Nigerian Aspergillus,

which produced fumonisin. This and the results are consistent with what the researcher reached, as he proved that the isolates were produced on PDA medium at a temperature of 25 °C continuously. Fig (4), Table (5).

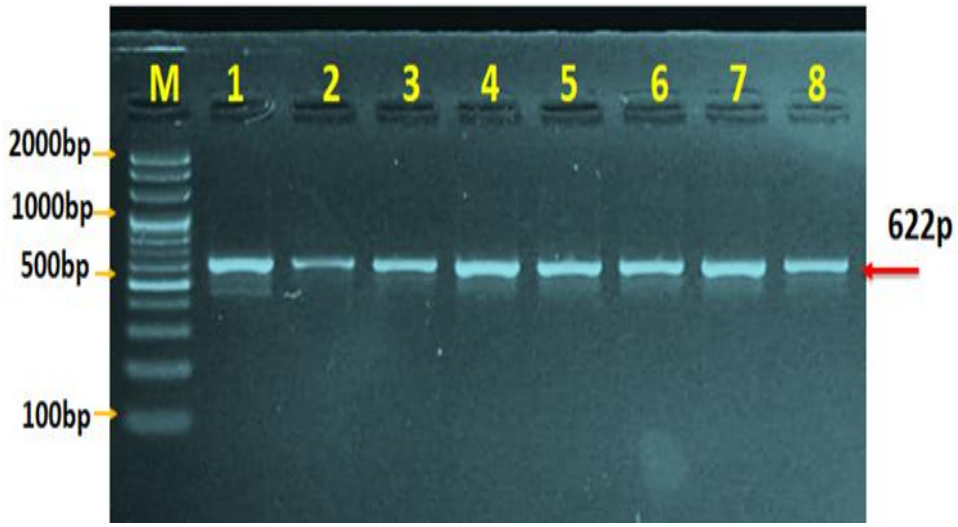

Fig (4): Agarose gel electrophoresis, which showed the degradation of the polymerase chain reaction product of the fum1 gene for fumonisin biosynthesis of Aspergillus niger isolates. The positive gene showed fumonisin biosynthesis for a gene of size 622bp.

Table (5) Genetic detection of the biosynthetic fumonisin fum1 of A. niger

Fumonisin biosynthetic fum1 gene	**Frequency**
Results	**N (%)**
Positive, *n* (%)	**8 (100.0%)**
Negative, *n* (%)	**(0%)**

Discussion

Initial culture of the fungus on SDA medium, where colonies of fungi appeared in dark black color, fast growing with divided and branched fungal hyphae, the vesicles are circular in shape, carried on a stand that is short and bears spherical conidia (Al-Sudani 2015; Chen et al., 2018). The cause of the spread of diseases of the genus Aspergullis spp. Because of the nature of the fungus, where its feeding is a throw on the host, where it is opportunistic, which leads to its spread widely, in addition to its small size and the production of many reproductive units, which facilitates its carrying, volatilization and spread, as it achieved with liquid plankton in the aerobic microbial community. Different areas of the body, especially diabetics. This is consistent with (Al-Amri, 2020) (Gregg et al., 2018) and its similar results with regard to the fungus Aspergullis niger, which had dominance because it was the highest frequency of the rest of the fungi that were isolated and diagnosed, but were excluded and included.

The reason for the dominance of Aspergillus is that these fungi have a high ability to adapt and reproduce and form many small spores that spread in the air and their high ability to withstand environmental conditions, including drought. This explains the varying numbers of isolated fungi (Ani et al., 2015) and the conditions in which The fungi grow in them due to temperature, humidity, and the speed of reproduction and spread in relation to the fungus. As for the special conditions of the patient, including low blood supply to the extremities and low rate of defensive immune cells or those with a defensive function, which is found in the opportunistic susceptibility of the fungus and its ability to secondary metabolites and enzymes (Flannigan et al ., 2011 Korbel and Spencer, 2015), and this is consistent with what Al-Husseinawi (2006)

found that the predominance of Aspergillus fungus was 25.0 in our study, which included isolating opportunistic fungi from different regions of the body, as the results reached by Minxi et al. 2019 indicated that aspergillosis is common in Type 2 diabetic patients without diabetic ketoacidosis, and drug resistance of Aspergillus spp. This also agrees with the results of our current study.

In order to distinguish them from the rest of the species, the cultivation traits and biochemical tests were adopted. The isolates were initially diagnosed based on morphological, cultural and microscopic characteristics, in addition to biochemical tests (Macfaddin, 2000), and the diagnosis, 18 SrRNA was investigated for the fungal diagnosis and 16 SrRNE for the final diagnosis. This is consistent with the findings of Mousavi et al., 2012; Eigl et al., 2017). All samples of *Aspergillus niger* isolates, which are proven in the table below, were subjected to the ability of the fungus to produce biofilms, where the results of electrophoresis for the alpha-1,3 glucan gene on the agarose gel showed 100% positive results for producing biofilms, and the gene size was 313 bp. *Aspergillus niger* has a high capacity. On the adhesion and production of biofilms, where biofilms developed with high production efficiency, and this agreed with what was stated by (Skowronek and Fiedurek, 2006; Maubon et al., 2006; Latgé and Chamilos G. 2020). These results are partially consistent with the findings of Frisvad et al., 2007; Medina et al., 2013;) and this study contrasted with the findings of (Frisvad et al., 2007) that fumonisin production was not demonstrated, although they tested only one isolate of *Aspergillus niger*.

Conclusion

One of the most important conclusions reached by our current study is that there are many and continuous infections in the diabetic foot due to more than one type of fungus, and that it was very resistant according to what was studied of the virulence factors.

Reference

Nordwall M, Hyllienmark L, Ludvigsson J. Early diabetic complications in a population of young patients with type 1 diabetes mellitus despite intensive treatment. *J Pediatr Endocrinol Metab.* 2009;19(1):45–54.

Chipkin, Stuart MD[§]; Lazar, Harold L. MD[*]; McDonnell, Marie M. MD[†]; Fitzgerald, Carmel NP[*]; Bliss, Caleb MA[‡]; Cabral, Howard PhD[‡]. Effects of Aggressive Versus Moderate Glycemic Control on Clinical Outcomes in Diabetic Coronary Artery Bypass Graft Patients. *Annals of Surgery* 254(3):p 458-464, September 2011.

World Health Organization (WHO): Definition, Diagnosis and classification of diabetes mellitus and complications. Part 1:Diagnosis and classification of diabetes mellitus. Department of Non-communicable Disease Surveillance, Geneva, 2015.

Perkins BA, Orszag A, Ngo M, Ng E, New P, Bril V. Prediction of incident diabetic neuropathy using the monofilament examination: a 4-year prospective study. Diabetes Care. 2010;33:1549–54.

Lebrun E, Tomic-Canic M, Kirsner RS. The role of surgical debridement in healing of diabetic foot ulcers. Wound Repair Regen. 2010;18:433–8.

Game FL, Hinchliffe RJ, Apelqvist J, et al. A systematic review of interventions to enhance the healing of chronic ulcers of the foot in diabetes. Diabetes Metab Res Rev. 2012;28(Suppl. 1):119–41.

Chen M, Xu Y, Hong N, *et al*. Epidemiology of fungal infections in China. *Front Med* 2018; 12: 58– 75.

Gregg EW, Cheng YJ, Srinivasan M, *et al*. Trends in cause-specific mortality among adults with and without diagnosed diabetes in the USA: an epidemiological analysis of linked national survey and vital statistics data. *Lancet* 2018; 391: 2430– 2440.

Korbel L, Spencer JD. Diabetes mellitus and infection: an evaluation of hospital utilization and management costs in the United States. *J Diabetes Complications* 2015; 29: 192– 195.

Minxi Lao, Chen Li, Jin Li, Dubo Chen, Meilin Ding, Yingying Gong, Opportunistic invasive fungal disease in patients with type 2 diabetes mellitus from Southern China: Clinical features and associated factors First published: 22 November 2019. https://doi.org/10.1111/jdi.13183

Eigl S., Hoenigl M., Spiess B., Heldt S., Prattes J., Neumeister P., Wolfler A., Rabensteiner J., Prueller F., Krause R., et al. Galactomannan testing and aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. *Med. Mycol.* 2017;55:528–534.

Maubon D, Park S, Tanguy M, Huerre M, Schmitt C, Prévost MC, et al. *AGS3*, an α(1–3)glucan synthase gene family member of *Aspergillus fumigatus*, modulates mycelium growth in the lungh of experimentally infected mice. *Fungal Genet Biol.* 2006;43:366–375.

Frisvad, J. C., Larsen, T. O., Thrane, U., Meijer, M., Varga, J., Samson,R. A., et al. (2011). Fumonisin and ochratoxin production in industrial Aspergillus niger strains. PLoS ONE 6:e23496. doi: 10.1371/journal.pone.0023496.

Medina A., Schmidt-Heydt M., Cárdenas-Chávez D.L., Parra R., Geisen R., Magan N. Integrating toxin gene expression, growth and fumonisin B1 and B2 production by a strain of *Fusarium verticillioides* under different environmental factors. *J. R. Soc. Interface.* 2013;10:20130320. doi: 10.1098/rsif.2013.0320.