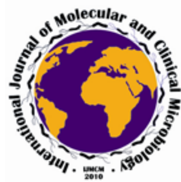

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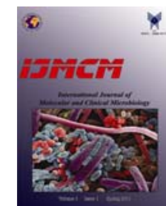

The study of Cumin oil essence on the function of FUM1 genes in *Fusarium verticillioides* by Molecular Analysis

Alireza Mokhtari[1*], Saeed hosseini[2], Shahnaz pourhossein[3], Nahid Soleimanifard[4]

1. PhD Bacteriology, Atiyeh Laboratory Complex, Fardis, Iran.
2. PhD Comparative Histology, Atiyeh Laboratory Complex, Fardis, Iran.
3. MSc, Biochemistry, Atiyeh Laboratory Complex, Fardis, Iran.
4. Department of Microbiology, School of Basic Sciences, Saveh Branch, Islamic Azad University, Saveh, Iran.

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ABSTRACT

Fusarium is one of the most important pathogens of cereals; the fungal infection has increased in recent years due to ecological changes and soil nitrogen content increase. In the current study the effect of cumin oil essence on *fusarium verticillioides* by molecular method was investigated. The study included 14 isolates, 11 isolates of *fusarium verticillioides* and 3 *Fusarium* non *verticillioides* samples (negative control) were tested. For testing MIC and MFC, the RPMI1640 culture in microplate was used. After a week, the cultured RNA samples using the RNA extraction kit of Fermentas company (Purification Mini Kit GeneJET Plant RNA) was extracted from fungal biomass and the RT-PCR test along with the *FUM1* gene was performed. The RT-PCR analysis displayed that *FUM1* gene was expressed in fungal samples that were not under the effects of oil essence, but the gene was not expressed in samples after the cumin impact and there was not any band in agarose gel. The fumonisin B1 toxin which was produced by the fungus *fusarium verticillioides* contains several genes. The gene *FUM1* was involved toxin gene synthesis pathway of this toxin as the most essential gene.

1. Introduction

Now in the developed communities the issue of food security has its special status. The consumers have become more sensitive to their health and the food contamination issues, especially to mycotoxins due to complications including cancer, genetic mutations, and teratogen, in the meantime, has found its specific position. This is due to the consumption of contaminated food of fungal, so that the consumer countries have put food safety in top priority and the technical aspects of this issue have overcome its economic aspects, in other words, mycotoxin pollution categories have been added to the indicators of the quality of the food. The 30-25˚C is the proper temperature for growing The Fungus. The heat tolerance is different among them and some species have the ability to grow at high temperatures (Doyle et al., 2013), (Samson and Pitt, 2013).

The Fungi grow in a wide range of pH and unlike bacteria the fungi grow in the medium with better growth conditions, nearly as acidic

*Corresponding author: Dr. Alireza Mokhtari
E-mail address: alm3370@yahoo.com

environment (pH=5-6) (Yazeed et al., 2011). Fungi grow in proper environmental conditions, produced metabolites some of which are toxic Carcinogenic, and teratogenic. Some other group also used in the pharmaceutical and fermentative industry (Hui et al., 2001), (Varga et al., 2009). To classify fungal food spoilage their appearance would also considered (Mogensen et al., 2009). Environmental temperature factors, pH, oxygen, water and light affected fungal growth respectively. The Fusarium species identification is difficult because of the difference between isolates (in terms of shape, size and color of colony) and the absence of the main building (without macroconidia) (De Silva et al., 2013). Important characteristics such as pigment on potato dextrose agar medium after 10 to 14 days of incubation, the diameter of the colonies on the above medium after 4 days of incubation in the dark at 25°C, microscopic morphology, the number of macroconidia, the number of macroconidia, the shape and production method of macroconidia in Fusarium species identification and isolation is much important. The most important fungi producing mycotoxin are three genera of Aspergillus, Fusarium and Penicillium. Environmental conditions of heat, moisture, insect damage and any stress to plants or crops, will lead to the production of mycotoxins in them. Although a variety of Fusarium are capable of producing fumonisin, but most of them are unknown, and they differ in terms of toxin production (Coulombe, 1993). Mycotoxicology or the science of mycotoxines began in 1960, following the X Disease outbreak in Great Britain turkeys that was related to the imported moldy grain from Brazil for feeding turkeys (Sargeant et al., 1998).

Some of the symptoms of Mycotoxicosis may reveals secondarily or may occur as the immunosuppression and provide the groundwork for other opportunistic diseases; it is difficult to distinguish the Mycotoxicosis specific symptoms (Hesseltine, 1999). So far, various species of *Fusarium verticillioides, Fusarium fujikuroi, Fusarium proliferatum* widely and other species (*Fusarium graminearum, Fusarium semitectum, Fusarium acuminatum,* etc.) with a lower incidence of rice grains have been isolated. Each of these species may produce a variety of mycotoxins. Fusarium species are different in terms of mycotoxins types. The fumonisin is the most important among these toxins which was identified in 1988 (Chandra Nayaka et al., 2009). The molecular specifications of regulatory genes involved in fumonisin biosynthesis plays an important role in identifying the toxin (Choi and Shim, 2008). The Ribosomal DNA discovered in the fungi nucleus including three replicate RNA genes (18S, 5.8S, 28S). Among these areas, the area coded ITS2 and ITS1 (internal transcribed spacer) composed of three distinct RNA, which is used to determine the genera (O'Donnell and Cigelnik, 1997). The RT-PCR method is seen as the highly sensitive test to detecting and quantifying the mRNA. Various studies show that the ITS region of fungal species varies greatly between them, and these areas have been investigated for the ability to produce toxins in fungi (Konietzny and Greiner, 2003). Vahid Rahjou and the collegues reported the 98% of the *Fusarium verticillioid* in 191 corn samples tested from eleven geographic regions of Iran by conducting a research that was carried out between 2005-2004, based on PCR test and determination of species and morphological characteristics by VER 1/2 5 / (Rahjoo et al., 2008).

Basically evaluating the antimicrobial effect of a substance based on laboratory procedure is being conducted for three purposes: Determining the strength of the antimicrobial substance in soluble form, Determining the minimum concentration of the substance in the body, Determining the sensitivity of a particular microbe into the substance densities, the Antimicrobial effect of the drugs measured by two methods: Conducting the tests to determining the drug dilution, Determining the MIC and MFC. In determining the MIC the minimum concentration is to preventing the growth of microorganisms by an inhibitor substance. These factors could vary in the ultimate test result in the next iterations or in different laboratories and different MIC for a chemical may be reported (Schwalbe et al., 2007).

These methods have been standardized by the CLSI. This test is conducted according to the NCCL - M38A2 standard (Pfaller et al., 2009a, Pfaller et al., 2011). Chemical compounds and the plant oil essence. Cumin has tannins, oils, resins and essential oils. The maximum cumin seeds chemical compounds contains: 1.8 grams

of water, 17.8 g protein, 3/22 grams of fat, 2/44 g carbohydrate, 5/10 grams of fiber, 6/7 grams of ash, 931 mg of calcium, 66 mg of iron, 366 mg of magnesium, phosphorus, 499 mg, 1788 mg, 168 mg sodium, 5 mg, 8 mg of ascorbic acid, 1 mg of thiamin, niacin 5 mg, 1270 international units of vitamin A and 375 kcal energy. The cumin seeds also contain 7% oil, 13% of resin and 5-2/5 % essential oil (Calo et al., 2015), (Messer et al., 2009). Cumin oil essence is located in the fruit (seed or grain) and inside the confined central cavity and it is obtained by distillation of the mashed fruits under influence of water vapor. The oil essence is a colorless liquid that turns into yellow and then brown colors and become sticky. The smell of the cumin is related to an aldehyde known as cuminol a very strong, almost harsh, and has a specific gravity of 0.93 – 0.91(Calo et al., 2015). It is soluble in 10 times its volume, 80% alcohol but with large quantities in more powerful alcohol. It is too much soluble in chloroform and ether. Aldehyde cuminic or cuminol, cuminol alcohol, Simonin, Alpha-pinene, beta-pinene, Gamatrpynn, phlandrene, cineole may, 1 and 3 Paramntadyn - 7-aL, 1 and 4 Paramntadyn -7-Al, camphene, myrcene, Myrtnal, Kapasymn, Dyhydrocomin Aldehyde, cariophiline, alpha terpineol, linalool, terpinene, pulegon are all the oil essence ingredients (Calo et al., 2015, Lee et al., 2007). Naini and the colleagues on their research in 2010, investigated the impact of five plant such as cumin essential oil on filamentous fungi by Fusarium the Cumin essential oil with consentration of 159 and 185.3 ug / m and has completely inhibited the growth of toxicogenic Fusarium non-toxin-causing Fusarium (Naeini et al., 2010). The purpose of this study effect of cumin oil essence on *fusarium verticillioides* by molecular method was investigated.

2. Materials and methods

2.1. Sample Preparation

In this study, this study was conducted on 14 species of isolates including nine species of *Fusarium verticillioid* fungi and three species of non-Toxin *Fusarium verticillioids* isolated from Iran Also, 2 isolates of the same type were approved from the Seed and Plant Research Center of Ministry of Jihad-e-Agriculture, Karaj, Iran. The production of fumonisin toxin was confirmed by HPLC method by the same center. The influence of cumin oil essence on isolated *Fusarium verticillioid* from Iran Through in vitro was determined by micro dilution MIC and MFC methods.

2.2. Culture fungi

The antifungal effects were also investigated, then, after fungi cultivation and RNA extraction, the RT-PCR test was done before and after the effect of oil essence so that the genetic effects of these fungi on two *ITS*, *FUM1* genes could be investigated. In order to studying the RNA and DNA and the tested isolates before and after the impact of oil essence, the special culture medium was prepared according to resources named induction of toxin production in this research (Fumonisin-inducing liquid medium) for more expressing the FUM_1 gene. The environment provided in broth method for broth and includes the following components:

Table 1. Fumonisin-inducing liquid medium (Jiménez et al., 2003)

Malt extract	Yeast extract	peptone	KH_2PO_4	$MgSO_4$-$7H_2O$	KCl	$ZnSO_4$-$7H_2O$	$CuSO_4$-$5H_2O$	fructose
0.5 g/L	1g/L	1 g/L	1 g/L	0.3 g/L	0.3 g/L	0.05 g/L	0.01 g/L	20 g/L

After preparing these environments in a broth form, the environment pH set on 2 so that the ability of the isolates to produce toxin according to Miller could be evaluated. After the environment autoclave, the culture of 7 days *Fusarium verticillioid* before the impact of oil essence was applied for culturing the induction of toxin production to the 10^6 fungal spores and the inoculation was done. Also among the samples after the MIC test and from the MIC well in case of growing, from the well before the MIC in which the growth of fungi is seen brought on each isolates on PDA separately, and then 106 fungal spores incubated to the to the fuminisin induction of toxin production. Then the incubation was implemented in a 200 rpm shaking incubator and temperature of 20 ° C for one week (López-Errasquín et al., 2007). This research has been executed on a total of over 14 isolates species, nine filamentous fungi

Fusarium verticillioid species and 3 *Fusarium verticillioid* species (as a control) which are isolated from Iran and been provided form the Mycology Center of Faculty of Veterinary Medicine Tehran University, also 2 standard approved isolates from the same species were prepared in which the amount of fumonisin toxin was confirmed by HPLC , from the Ministry of Agriculture Research center seeds and seedlings.

2.3. Antifungal effects

In order to determine the MIC at the beginning 100 ml of RPMI1640 medium was added with eight-channel pipette to the 96 well plate, Then a Soluble substance of 100 µl Cumin to the first well from the left was added and mixed well with the environment, In the following 100 ml was picked up from the first well and transferred to the second well and the½ dilution of essence was prepared in each well as the same way 100 µl essence was extracted from the tenth well. The well of 11 and 12 do not have any essence. Then 100 µl of standard suspension was added to each well in order that the soluble final volume of 200 µl is created with the different concentration of oil essence. The well of number 11 containing 100 µl of the environment and 100 µl fungal suspensions as a positive control environment or GC (Growth control) and the well of number 12 that contains the medium culture is considered as a negative control or SC (Sterility control) was considered. This test was performed for every isolates in horizontal row with two iterations (Cuenca-Estrella et al., 2010). The plates were incubated for 48 h at 35°C. After this time the plates were brought out from the incubator and were read visually and by Elisa reader machine at 540 nm. The well which Inhibit the growth of fungi was regarded as MIC. In the visual method a general rule was used to displaying the growth rate according to the guide, of five to zero scores (lack of clear growth), score of 1(25% growth), scores of 2 (50 percent), scores of 3 (75 percent), scores of 4 (100% growth) compared with control holes, which 100%, growth respectively. In this test the dilution of 100%the fungal growth inhabitation was considered as MIC90. Using the Elisa reader machine the lowest concentration equal to the MIC90 will be the test result (Cuenca-Estrella et al., 2010). The MFC is defined as the lowest concentration of an antifungal action where the concentrations of 99.9% of the microorganisms are killed. To determining the MFC, using a sampler, the MIC well and the well after MIC which has growth and well with higher levels of oil essence before the MIC the 50 µl were brought to the PDA medium and after cultivation, it was incubated for a week, After this time any dilution which prevent the fungal perfect growth and in the case of high difference between these two ,The fungus is resistant to the lethal action and if the difference between these two is low it shows the sensitivity of the isolates to the oil essence and it naturally doesn't need not to prescribe anti-fungal , to eliminate it. (Cuenca-Estrella et al., 2010, Espinel-Ingroff et al., 2002).

2.4. Extraction

For DNA extraction the *Fusarium verticillioid* was used from the commercial kit of Fermentas Company (Thermoscientific. USA) called DNA Purification Mini Kit GeneJET ™ Plant Genomic. For RNA extraction the filamentous fungi fusarium verticillioides used from the commercial kit Fermentas company (Thermoscientific. USA) called RNA Purification Mini Kit d GeneJET Plant (Fermentas) was used. The PCR experiments was conducted on extracted RNA to assess DNA contamination. In order to perform this test the RNA samples that were affected by DNase with amount of lµ5 as well as on DNA samples extracted with the kit with amount of lµ5 and the PCR tests with defined primers were used, so the absence of DNA be ensured. Converting the RNA to cDNA by RevertAid First Strand cDNA kit Synthesis Kit of Fermentas Company. After fungal isolates DNA extraction by ITS primer that the sequences are listed in the table 2 the PCR test was done in order that the genus is being approved. This primer is being performed for separating the Fusarium among the similar fungi, such as Aspergillus, and Alternaria. This primer bands is detected at 431 bp (Karthikeyan et al., 2011). In this reaction the Aspergillus DNA was used as a negative control. The test is performed in a volume of 25-ml.

Upon confirmation of Fusarium species with ITS primers, samples were amplified by VERTF1, VERTF2 sequence mentioned in the Tablet, the PCR test was conducted to final

approval of all Fusarium isolates be confirmed in terms of the genus and species of *Fusarium verticillioid* (Karthikeyan et al., 2011). In this test the Fusarium non verticillioides was used as a negative control.

This primer is being applied to differentiate between the species which are able to produce fumosinin species and species that are not able to produce fumosinin. This primer is from the coded Inter Generic Spacer area (IGS) in rDNA (Karthikeyan et al., 2011).

2.5. Electrophoresis

The final PCR product was observed in accordance with the clause on 2.1% agarose gel and after coloring electrophoresis with ethidium bromide by Doc Gel machine. The studying of the FUM1 gene expression to controlling the expression in DNA samples extracted from isolates. the DNA samples extracted from the *Fusarium verticillioid* which have been cultured under the conditions mentioned in paragraph - in the induction of toxin culture medium and then their DNA has been extracted, were used to evaluate the production of fumonisin toxins of Polyketid synthase (PKS) FUM1 genes (Sreenivasa et al., 2011). This study was experimental and data was processed using SPSS software version 19 and descriptive statistical methods. Student t-test was used to compare the incubation times of 96 home microplates and did not show a significant level ($P \leq 0.05$).

Table 2. Nucleotide sequences of primers used in the PCR test to detect Fusarium (Bluhm et al., 2004, Sreenivasa et al., 2011)

Primer	Target gene	Length	Sequence (5´- 3´)	Amplification Product (bp)
ITS- f	*ITS*	24	5´- AAC TCC CAA ACC CCT GTG AAC ATA-3'	431
ITS- R	*ITS*	17	5´- TTT AAC GGC GTG GCC GC-3'	431

Table 3. The Nucleotide sequences of primer used in the PCR test to identify the genus and species of fusarium verticillioides (Karthikeyan et al., 2011)

Primer	Target gene	Length	Sequence (5´- 3´)	Amplification Product (bp)
$VERTF_1$	*IGS*	20	5´- GCG GGA ATT CAA AAG TGG CC-3'	400
$VERTF_2$	*IGS*	20	5´- GAG GGC GCG AAA CGG ATC GG-3'	400

Table 4. The PCR reaction test plan for detection of *Fusarium verticillioid* (Karthikeyan et al., 2011)

Initial denaturation	**95° C , 3 min**
Amplification (32 cycles)	**Denaturation : 95° C , 1 min** **Annealing 60° C , 1 min** **Extension : 72° C , 3 min**
Final extention	**72° C , 5 min**

Table 5. The nucleotide sequence used for gene expression FUM1 (Bluhm et al., 2004 ,Sreenivasa et al., 2011)

Primer	Target gene	Length	Sequence (5´- 3´)	Amplification Product (bp)
FUM_1-F	*FUM_1*	20	5´- CCA TCA CAG TGG GAC ACA GT-3'	183
FUM_1-R	*FUM_1*	22	5´- CGT ATC GTC AGC ATG ATG TAG C-3'	183

3. Results

The susceptibility testing results and the MFC and MIC results: the fungi MIC were determined using micro dilution methods. After that, the extra culture tubes were perfumed to determine MFC, the test results interpretation is made in the following reference. In order to assessing the antifungal effects of Cumin oil essence; the 2% G RPMI 1640 medium was used. This means that after preparing the environment with 2% glucose and buffer MOPS, the media culture was filtered and placed in the refrigerator until it will be consumed. The Plates were incubated at 35 degrees Celsius for 48 hours. For each of the microorganisms a positive control well (GC) and negative (SC) was considered.

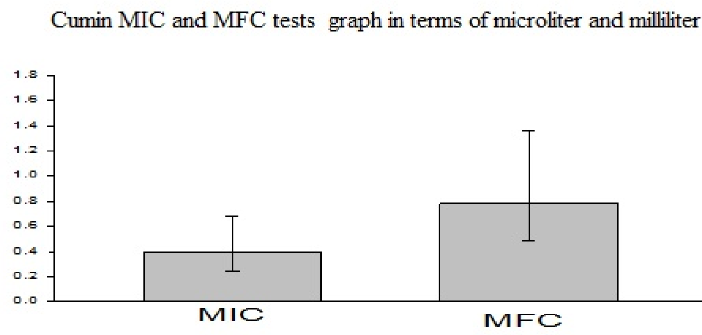
The MIC test results show the high influence of Cumin oil essence on *Fusarium verticillioid*. It is noteworthy in this study that the soluble specification of Cumin oil essence in water solution requires no type of solvent such as DMSO so it is easily dissolved in the liquid medium. In all MIC and MFC experiments that have been performed twice, it was found that the effects of the oil on the tests presented the same results. The MIC test results the microplate method of all samples among well number 7 to 9 was observed. Also on the table 6 the frequency of 11 samples tested *Fusarium verticillioid* results in MIC and MFC been mentioned. In this table, the highest frequency of testing MIC concentration of 0.390 µl/ml with (54.5%) and least frequent in concentration of µl / ml 0.195 (18.2%) was obtained. in order to determining the MFC of these isolates, after determining the MIC and determining the well, the dilutions before and after MIC specification, transferred to the PDA agar environments and cultured and after the time incubation the dilutions which have not been grown on agar plate reported as MFC. The MFC test results between 8-6 well obtained and similar results were observed in both times. In MFC tests the highest frequency rate in the concentration of 0.781 µl/ml with (54.5%) and least frequent in concentration of µl /ml 0.390 (18.2%) was obtained.

Conidia for microscopic examination of samples that have been affected by the oil, the samples grown on plates to determine the MIC and MFC and colonies appeared on the plates-were used. After preparing the slides in the vicinity of Lacthophenol cotton blue solution, select deformed samples in comparison with normal fungi clearly (Picture-1) is visible.

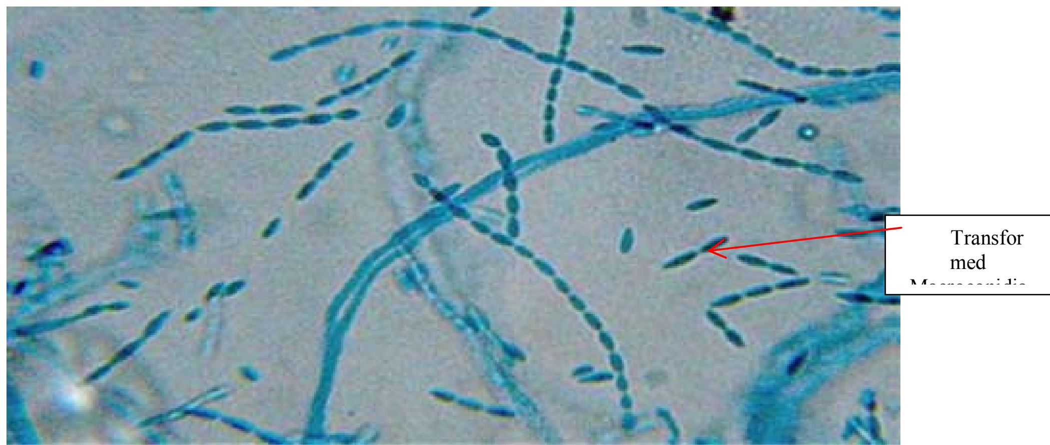
Samples tested after approval and confirmation of the genus Fusarium fungi example, fungi *Fusarium verticillioid* to confirm the toxicogenic gene PCR test was performed 1,2 VERTF. These gene products visible in Fig below 400 bp established clearly visible. At first, the experiment was conducted with 14 isolates of the three species of non-toxicogenic were detected. Then the test was also conducted with 11 isolates separately.

Table 6. The MIC and MFC test results of Cumin oil essence in terms of (µl / ml)

Sample number	F_4	F_5	F_9	F_{11}	F_{13}	F_{14}	F_{16}	F_{17}	F_{18}	F_{R200}	F_{R201}
MIC	0.195	0.781	0.781	0.390	0.390	0.390	0.781	0.390	0.195	0.390	0.390
MFC	0.390	1.56	1.56	0.781	0.781	0.781	1.56	0.781	0.390	0.781	0.781



The chart 1. results of microscopic morphology *Fusarium Verticillioides* affected by oil



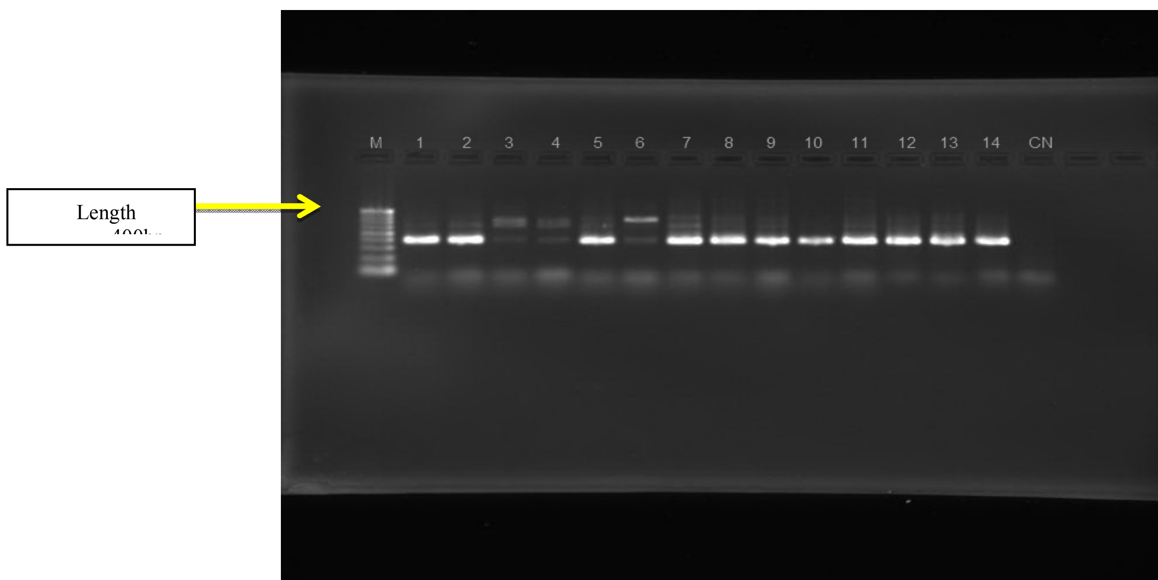


Fig 2. 1,2 100bp, 1,2,5,7,8,9,10,11,12 row samples *Fusarium verticillioid* toxicogenic, rows 3,4,6-causing *Fusarium verticillioid* non- toxicogenic, rows 13 and 14 of *Fusarium verticillioid* (positive control), CN (negative control)VERTF gene agarose gel to confirm the 14 fungal species: in order from left to right: row M: Marker.

4. Discussion

The Fusarium fungi is widely distributed and it has been reported in the fields of grain abundantly. In the meantime, those spices of Fusarium producing toxin are of importance due to contamination of food and animal and human health threats. Identifying these fungi in determining the amount of products affected in different ways plays an important role in controlling and combating against the toxin producing Fusarium including *Fusarium verticillioid*. Now a days, the diagnostic procedures become more common in addition to routine diagnostic methods including culture, examining morphological specification, molecular methods due to high speed and more accurate identification as well as assessing the details of genes producing -toxin and been more efficient. The evaluation studies in Iran and elsewhere suggests that there is a high incidence of Fusarium, especially *Fusarium verticillioid*. Khosravi and the colleagues isolated Fusarium in 6% samples of grain corn, barley and corn silage and animal feed in 2008 in Qom (Khosravi et al., 2008). Vahid Rahjou and the collegues reported the 98% of the *Fusarium verticillioid* in 191 corn samples tested from eleven geographic regions of Iran by conducting a research that was carried out between 2005-2004, based on PCR test and determination of species and morphological characteristics by VER 1/2 5 / (Rahjoo et al., 2008). Sreenivasa and the colleagues evaluate the Fusarium species producing Fumonisin in freshly harvested corn grain by PCR method in 2006 and 7 isolates of the fungus were detected (Sreenivasa et al., 2011). Juardo and the collegues suggested and applied the PCR strategy in 2006 for rapid detection of Fumonisin in animal food production that causes cancer in animals in India (Jurado et al., 2008). Heidi and the colleagues have identified Fusarium species that generated the fumonisin toxin by PCR method in 2011 in Egypt. In this study 2 genes were applied that 21 isolates were identified and isolated from those toxins producing fungi in animal feeds. (Heidy et al., 2011). Leslie and colleagues applied both morphological and molecular characteristic to identifying the Fusarium producing Fumonisin in 2006 in Iowa of America (Leslie et al., 2008). Our results confirmed that all of the tested isolates were susceptible to the essential oil, indicating a significant reduction in the growth of fungal isolates. The rate of growth reduction was directly proportional to the concentrations of tested oil in the medium. In fact, higher concentrations of the oil led to lower fungal developments, representing 54.5% of the strains inhibited at 0.390 µl.ml. The oil essence is perceived as the Medicinal herbs ingredients which are medically known. Cumin oil essence has a long history in the treatment of diseases. So that the Iran country holds a large share of global production and export of cumin, the cultivation of this product, in addition to Iran, is common in many countries around the world but countries such as Iran, India and Egypt has their special place in the field of manufacturing and exporting the plant. The MIC and the MFC tests displayed that the oil essence has strong antifungal effects. The effect has been also proven in other studies on the *Fusarium verticillioid* and the other fungi as well. The fungi in other researchers' results were also affected by other medical herbs oil essence and results of this study showed effectiveness of these oil essences in inhibiting the growth of *Fusarium verticillioid*. Khosravi and the colleagues investigated the , the impact of medicinal plant oil essence including cumin on two types of fungi *Aspergillus fumigatus* and *Aspergillus flavus* in 2011 and found that it has a very strong antifungal activity with MIC90 of about mg / ml 2-5 / 1 (Khosravi et al., 2011). Khosravi and minoueyan in an article in 1388 studied the impact of Cumin, Nigella, Ziziphora tenuior oil essence on, *Aspergillus flavus* and *Aspergillus fumigatus*. In this study, Cumin oil essence has been the greatest antifungal impact at the lowest reported quantity. The study also pointed to the fact that main cumin oil essence ingredients are Parasymol, Symin, Pynin, cineol and linalool (Khosravi et al., 2011). Naini and the colleagues on their research in 2010, investigated the impact of five plant such as cumin essential oil on filamentous fungi by macrodilution method including non – toxicogenic Fusarium (*Fusarium oxysporum* and *Fusarium solani*) and toxicogenic Fusarium (*Fusarium verticillioides, Fusarium Pouyeh* and *Fusarium equiesty*). In this study, the Cumin essential oil with consentration of 159 and 185.3 ug / m and has completely inhibited the

growth of toxicogenic Fusarium non-toxin-causing Fusarium (Naeini et al., 2010). Shokri and the colleagues in 2011 have studied the impact of two types of essential oil (*Zataria multiflora*, *Geranium pelargonium*) on 4 fungal toxin-causing spices and offered desirable results from the impact of the two plant oil essence on a number of toxin-causing fungi, including *F.verticillioides* (Shokri et al., 2011). In addition, *C. cyminum* oil exhibited a remarkable fungicidal activity against the fungal isolates with MFC values ranging from 0.390 to 1.56 µl.ml. In a study conducted in 2010 by Omar Arturk the effects of alcoholic extract of 41 herbs such as cumin on different microorganisms such as *Aspergillus niger* and *Candida albicans* was studied, among those plants the Cumin had a very good influence on the growth of these microorganisms (Ernst, 2005). In evaluating and comparing the results between the MIC and MFC, results indicate that, there is a short distance among the lowest dilution inhibiting the growth and the fungicidal dilution. The comparison between the incubation time of 48 hours and 72 hours of microplate in the incubator and observing no difference between the two times, can be confirmed that The effective and active oil essence ingredients in the environment and has left its own impact from the very early hours and increasing incubation time has no effect on the antifungal activity of essential oil. Sunita Bansod and Mahendra Rai in a research they have conducted in 2008 on the function of anti-fungal 15 essential oils from Indian medicinal plants species such as *Aspergillus fumigatus* and *Aspergillus niger* human pathogens, reported that cumin oil essence has weak antifungal activity compared to other essential oils such as eucalyptus globulus herbs (Bansod and Rai, 2008). Today using biological methods or the use of natural ingredients or ingredients of medicinal plants in controlling the pests is growing because of the chemistry side effects. Siddaiah Chandra Nayaka published a report in Nuremberg in 2009 ,according to the report, the corn seeds were sprayed with *Pseudomonas fluorescent* bacteria along with the control group that have not been sprayed with these bacteria, they announced the sprayed seeds generate more resistance compared to the control group compared to against *Fusarium verticillioid* and subsequently fumonisin. This study identifies an important role and low-cost of biocontrol fungi in prevention of toxin-causing fungi cereal pollution (Chandra Nayaka et al., 2009). Nowadays the use of molecular methods to identifying the toxin-producing fungi and toxins and evaluating the expression of genes producing toxins, effective factors on gene expression has been increased. In the confirmation of the genus and fungi species with *ITS* and *VERTF* genes were reported with 100% effectiveness. Gonzalez and the colleagues in 2004 mentioned the using of genetic indicators for the analysis of changes in the producing Fumonisin gene sequence of the *Fusarium verticillioid* species (Mulé et al., 2005). In another study of Gonzalez and the collegues in 2004 pointed to the using different molecular techniques to identifying the Fusarium species especially those toxin-producing such as *Fusarium verticillioid* with various primers (Mulé et al., 2005). Sreenivasa and the collegues in 2006 have identified the properties of different Fusarium the based on the ITS gene and the Fusarium generating fumosinin based on *FUM1* genes (Sreenivasa et al., 2011). Karthikeyan and the collegues have recognized the fungi producing the *Fusarium verticillioid* toxin based coded genes. District Inter Generic Spacer(IGS) called VERTF in their study that was published in 2011 (Karthikeyan et al., 2011). In this study also, the gene was used and 100% of its effectiveness in detecting fungal species were demonstrated. Also investigating the *FUM1* gene expression in terms of temperature and incubation time also indicate that the impact of these factors on gene expression and the transcription level. Gonzalez and the colleagues also in an article in 2008 have examined the relationship between stress, temperature and growth in the *FUM1* gene expression in isolated *Fusarium verticillioid* species in Spain by RT-PCR method. This study confirmed that low temperature and water stress reduced fungal growth in throughput. Water stress could lead to an increased level of FUM1 gene transcription (Jurado et al., 2008). Gonzalez and the colleagues in 2010 have conducted a research in the city of Madrid, Spain, in which the subtracting influence of environment on growth condition and isolated *FUM1* genes biosynthesis from corn infected with *Fusarium verticillioid* and *Fusarium Proliferatum*, and also the temperature effects in Invitro condition on

fungal growth and the *FUM1* gene expression levels were determined by Real Time RT-PCR. In this study it was found that the *Fusarium verticillioid* grew at higher temperatures but the *FUM1* gene expression induced in above 20 ° (Marin et al., 2010). Keller and the colleagues reported in 1997 that, the amount of oxygen and pH of as the effective factors for toxin production (Keller et al., 1997).Miller's announced clearly the desired pH and the optimum for toxin production in vitro pH = 2 in his own research (Miller, 2001). Shim and the colleagues published their study in 1991 insist on the role of nitrogen in expression levels of genes involved in the biosynthesis of B1toxins (Shim and Woloshuk, 1999). Richard Baird identified the FUM1 gene identification between 16S-23S rRNA region among the toxin-producing and non-toxin producing species on Fusarium producing verticillioides in his study of 2008. It also noted that the fomunisin generates in rice medium of 92% of tested *Fusarium verticillioid* species compare to the liquid medium (laboratory synthesis) in 37% of the spices (Baird et al., 2008). In order to evaluate the effect of *C.cyminum* oil on expression of *ITS* and *FUM1* genes encoding proteins involved in fumonisin biosynthesis, *F.verticillioides* isolates were cultured on fumonisin-inducing liquid medium in presence of *C. cyminum* oil (0.195 and 0.390 µl.ml-1) at 20°C for 7 days. The fungal mycelia were separated by filtration, then total RNA was extracted and the expression of *ITS* and *FUM1* genes was evaluated by RT-PCR. The results showed that *C. cyminum* oil completely inhibited the expression of *FUM1* gene in both concentrations dose-dependently. Based on the statistical analyses, reduction in the expression of fumonisin biosynthetic genes was significant only for FUM1 gene ($p<0.05$), while no effect was observed on ITS gene. Lopez-Errasquin et al. (2007) demonstrated a very good linear regression between FUM1 transcript levels and fumonisin production using RT-PCR. Despite the known antifungal activity of *C.cyminum*, no information has been reported about its effect on fumonisin biosynthesis in gene expression level. In a study by Khosravi et al. (2011), *C. cyminum* essential oil showed significant reductions in values of 94.2% for aflatoxin (AF) B1, 100% for AFB2, 98.9% for AFG1, 100% for AFG2, and 97.5% for total aflatoxin. Another study by Bluma et al. (2008).

Conclusion

But in the study of this toxin in all tested samples that had been inoculated synthetic medium, the production of which were confirmed by gene expression FUM1 was took place. Richard Baird also points out that the genetic variations among the Fusarium species occurring frequently. Applying the ITS gene in identifying the Fusarium species have been reported successfully, the study also proved this point (Baird et al., 2008). The point was observed in the present study as well and it emphasis on the proof of this category. He also stressed on the fact that the high pH is seen as of the limiting factors for generating toxin. In this study, we have tried by taking advantage of medicinal herbs such as cumin according to age and its impact take however brief but valuable steps instead of using chemical methods to combat pests of crops that have a major role in human and animal food chain. All together, these results indicated that *C. cyminum* may be employed successfully as a good candidate in controlling toxigenic *F.verticillioides* on food and feed and subsequent contamination with fumonisin in practice.

Refereces

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