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# PATHOGENICITY OF FUSARIUM VERTICILLOIDES ON MAIZE RELATED TO DETECTABLE FUMONISIN B1

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## ABSTRACT

Aggressiveness of 10 *Fusarium verticilloides* isolates originated from naturally infected maize grains were studied towards susceptible cultivar ,Three Way Cross(TWC-310) seedling. Isolates differentiation has been done based on pathogenicity parameters as: a percentage of non-emerged seedlings, survival plants, plant vigour and disease severity. All tested isolates expressed various degrees of pathogenic capabilities as slightly, moderately and highly virulent. Percentage of survival plants and vigour were reduced, while disease severity ratio increased from 2.7 - 51.6%. Fumonisin production can play a role not only in plant pathogenicity but also in pathogen aggressiveness. The study suggested to correlate between the ability of examined isolates to produce that mycotoxin *in vitro* with their phytotoxicity behaviour. Isolates have been screened if some or all can synthesize fumonisin B1, whereas relatively different levels were detectable ranging from (400-1.680 µg/ mL). So, positive relationship between the aggressiveness of tested *Fusariu*m isolates and their ability to produce this metabolite *in vitro*.
**Keywords:** *F. verticilloides*, maize, fumonisin B1.

## INTRODUCTION

*Fusarium verticilloides* (Sacc.) Nirenberg (Syn. *Fusarium moniliforme Scheldon*) is a widely distributed pathogen of maize (*Zea mays* L.) and many other plant species as banana, cowpea, fig, sorghum, tomato, rice and wheat (Bacon *et al*., 1994, chulze *et al*. 1996 and Jardine and Leslie 1999). *Fusarium* isolates recovered from maize are commonly associated with root, ear, stalk rots and soil inhibitant. This pathogen may be seed borne endophytes or may infect the plant causing poorly stands during various stages of plant growth.

The epidemiological studies show that *F. verticilloides* has one comparative advantage over other species of the genus *Fusarium*, especially in relation to *F. graminearum* Schwabe, as it requires a greater range of temperature and humidity for its development, hence the competitiveness of the fungus did not change in different environments. So, these this species colonise the plant tissue and remain at the dormant stage or at the endophytic stage, as long as the tissue is healthy and active (Munkvold *et al*., 1997 and Wicklow *et al*., 1998).

It is difficult to discuss with certainly the role of *F. verticilloides* in the etiology of seedling diseases, as seedling infection and the disease development are produced by the seed borne disease or a infested soil, and depend on the temperature during the maize growing season. *F. verticilloides* does not affect seed germination, but it affects the thickness, height, weight and leaf length of seedlings developed from infected seeds. On the other

hand, some strains of this fungus can even stimulate an earlier growth of seedlings.

The frequency occurrence of *Fusarium* species is not always correlated with their effect on seed germination, the following species are most often isolated from maize grains: *F. verticilliodes* 50.2%, *F. proliferatum* (Matsushima) Nirenberg 7.9%. However, germination of seeds infected with *F. subglutinans*, *F. proliferatum* and *F. verticilloides* was amounted to 15.3%, 23.4% and 32.6% respectively. A study reported that all ten isolates of *F. verticilloides* originated from maize and wheat, expressed a different degree of pathogenicity of corn seedlings, by reducing the plant survival rate and vigour. These results point out to a high genetic diversity of fungal population ( Krnjaja *et a*l., 2007).

*F. moniliforme* can produce noxious secondary metabolites such as fumonisins, fusaric acid, fusarins, and moniliformin, with additional compounds still remaining unidentified (Thiel *et al*., 1986, Nelson *et al*., 1991. and Leslie *et al*., 1992). Although Cvetnic *et al*., 2005 found that some *F. verticilloides* isolates from agricultural regions of Croatia can produce (ZEN) zearalenone, Jajic *et al*., 2007 in Serbia reported that corn kernels infected by *F. verticilloides* isolates which by contaminated harvested corn with rather high quantities of (DON) deoxynivalenol (200 – 2.460 µg/kg), and during submerged cultivation of fungal isolates in liquid media (GPY and SPY) all 4 tested isolates biosynthesized T-2 toxin (80 – 240 µg/L).

The fumonisins are the most prominent class of mycotoxins produced by *F. moniliforme*. The role of this toxins group in plant pathogenesis is not clear. Desjardins *et al*., 1995 found that aggressiveness, as assessed by maize seedling blight, was correlated with the fungal ability to produce high levels of fumonisin *in vitro*. Purified fumonisins can cause necrosis and other disease symptoms in maize seedling and to maize callus and can induce apoptosis in tomato protoplasts and cultured animal cells Lamprecht *et al*., 1994. In contrast, Abbas and Boyette, 1992, reported no phytotoxicity from fumonisin at concentrations up to 1.000 µg/mL, to 7 to 10 days old corn plants. Others in the field, location, hybrid and environmental conditions all impact fumonisin contamination levels (Shelby *et al*., 1994). Another study mentioned that the optimal production of fumonisin B1, B2 and B3 was by using a highly toxigenic strain of *F. verticilloides* (NRRL- 3428) isolated from feeds and when the strain was grown on cracked corn with 50% water content at 21°C during 5 weeks, this allowed the production of 3- 4 g of fumonisin B1 per kg of culture material (Bailly *et al*., 2005).

Others, found no significant differences in aggressiveness towards mature sorghum between strains of *F. moniliforme* and *F. thapsinum* (Jardine and Leslie, 1992 and Jardine and Leslie, 1999).

Fumonisins characterized as tricarboxylated amino polyalcohol, similar to esfingosins, they cause toxicity, inducing cerebral lesion- LEME in horses, lung edema in swines, imunodepression in poultry and hepatic carcinogenicity in rates (Marasas *et al*., 1988 and Galvano *et al*., 1997). In humans, the natural occurrence was associated with oesophageal cancer in South Africa and China (Wang *et al*., 1995). The food and Drug Administration currently is reviewing a draft of maximum allowable levels of

total fumonisin in corn products destined for human or animal consumption. For human consumption, the allowable levels are 2- 4 ppm for different corn food products. For animal consumption, the levels are 5 – 100 ppm for different animal feeds (U.S. FDA/ CFSAN 2001). Recommended maximum fumonisin B1 concentrations of 5- 50 ppm for different livestock feeds were adopted by the American Association of Veterinary Laboratory Diagnosticians.

Although the literature describes eight fumonisins. Only FB1, FB2 and FB3 have been detected naturally in corn in the pre-drying step of harvested corn. Natural fungal and fumonisin contamination levels were evaluated in freshly harvested corn, observing positive correlation with damage kernels percentage. FB1 and FB2 levels ranged from 0.57- 20.38 µg/g in kernels, while in the damaged kernels ranged from 68.96 – 336.38 µg/g, but non- significant correlation among the fumonisin levels and protein or lipid content of kernels (Ono *et al*., 2006). In Iran, 38 corn samples were analyzed using HPLC, and all except one showed high level of FB1 contamination ranged between 1.19 and 12.95 mg/kg (Yazdanpanah *et al*., 2005). Some of the existing methods for detecting fumonisin in corn and *F. verticilloides* cultural filtrate include liquid chromatography, gas chromatography mass spectroscopy, thin layer chromatography, capillary zone electrophoresis, immunosorbent assays, immunoaffinity column assays and high- performance liquid chromatography (Schaafsma *et al*., 1998, Yu and Chu, 1998 and Dowell *et al*., 2002).

Our objective in this study is to determine if the examined isolates of *F. moniliforme* differed in their aggressiveness towards corn seedlings and if these differences, if any, are correlated with the ability to produce fumonisin B1 *in vitro*.

## MATERIALS AND METHODS

**Isolates collection:**

Ten isolates of *F. verticilliodes* originated from grains of commercial maize hybrids collected from different governorates in Egypt (Three from Beheira, three from Ismailia, three from Garbia and one received from Kafr EL-Sheikh, respectively. Isolates were identified using of the procedure outlined by Nelson *et al.*, (1983) and Burgess *et al.,* (1994).

**Pathogenicity Tests:**

Petri dishes with two layer filter paper and covered with sterile quartz sand, instead of soil have been used for the development of the fungus and artificial inoculation of (TWC-310) corn grains. Surface- sterilized (45 maize grains/ isolate) with sodium hypochlorite, were inoculated in dishes (100 mm) with 30mL of spore suspension (2-3 x $10^6$ spore $mL^{-1}$). Fungal spore suspension was prepared from 7-10 old isolates cultured on PDA(potato dextrose agar) medium at room temperature. Inoculated and non-inoculated (control) maize grains were incubated at 22°C for two days and at 10°C for another three days, and then planted into flats with sterile quartz sand (40 X 18 X 16 cm), watered and incubated at 24 - 26°C for two weeks and the following was determined:

Degree of pathogenicity, length (cm) and dry weight (g) of seedling roots and epicotyls. In this experiment, the degree of pathogenicity was detected basis of the percentage of non-emerged seedlings (%), which was an outcome of seeds that had never germinated, and germinated seeds with completely rotted roots and shoots. According to this parameter, isolates were classified into five categories based on the scale reported by Maçka (1989) (Table 1). Modified inoculation test described by Molot and Simone (1967).

**Table 1: Pathogenicity estimation scale of *F. verticillioides* isolates.**

| Percentage of non-emerged seedlings | Degree of pathogenicity |
|---|---|
| 0 -10% | Non pathogenic |
| 11 – 20% | Very low pathogenic |
| 21 – 40% | Low pathogenic |
| 41 – 60% | Moderatly pathogenic |
| 61 – 80% | Highly pathogenic |
| 81 – 100% | Very highly pathogenic |

Disease severity was also used as a measurement of pathogenicity of tested isolates, and was rated by six-class scale, which by 0 = healthy root and epicotyl, and 5= non germinated grains or completely rotted root and shoot. The length of each seedling from the grain attachment site to the top of the longest root and leaves was measured (cm). The detached root and epicotyl per replicate were dried at 60°C for 24 hours and then, their weights (g) were measured. Means were compared by Duncan›s multiple range test.

**Fumonisin production assay:**

Isolates were cultured in corn culture by inoculating 2 mL ($10^6$ spore/mL) of each tested isolate on the surface of 100 g of ground corn, previously humidified with 100 mL of distilled water and autoclaved for 30 min. After incubation at 25°C for 30 days, the culture was solvent treated as described below and FB1 analyzed by HPLC.

**Fumonisin Extraction:**

Cleaning of the crude culture was conducted by using 400 mL of acetone : chloroform 75 : 25 (V/V), by overnight agitation of the sample at 180 rpm at 25°C. The extracted material was filtered through Whattman no.1 filter paper, and the culture residue cleaned again with acetone : chloroform 75 : 25. The solid residue was evaporated in air circulation.

**Determination of fumonisin by HPLC:**

The fumonisin was determined using the method of Shephared *et al*., (1990), modified by Ueno *et al.,* (1993). One mL of the culture filterate was clarified in Sep Pak Accele Plus QMA Cartridges, previously conditioned with 6 mL methanol : water at 3 : 1 (V/V) ratio, followed by elution with 3 mL methanol. The toxin was eluted with 10 mL ethanol with 0.5% acetic acid, evaporated at 40°C and re-suspended in 1mL of methanol. After drying, 2 mL of methanol : water (3 : 1) was added and evaporated under nitrogen at 50°C. For analysis, it was suspended in 800 µL of methanol : water (3 : 1) and a

200 µL aliquot was dried under nitrogen. After derivatization with 225 µL of (OPA) ortho-phtaldehyde (40 mg orthphtaldehyde, 1 mL methanol, 5 mL 0.1 M sodium borate and 50 µL 2-mercaptoethanol), the analysis was carried out in isocratic HPLC (Shimadzu LC-10AD) using reverse phase $C_{18}$column (250 X 4.6 mm), with 5 µm of Supelco·s nucleosil. The mobil phase consisted of methanol : sodium phosphate 0.1 M (80 : 20) adjusted to PH 3.3. The equipment was conditioned to a flow of 1mL/min. with wave length of excitation and emission of 335 nm and 450 nm (Shimadzu F 535), respectively.Quantification was done by measuring peack area and comparing with standard calibration curve . The mean retention time was 7.5 min. for FB1 .

O-phthaldehyde (OPA) was added to both the standards and samples prior to HPLC, since fumonisins, are unable to absorb either UV or visible light and are unable to fluoresce. OPA derives the fluorescent products from the fumonisins (Sydenham *etal.,* 1992). OPA 225 µL was added to 25 µL of the standard and 10 µL was injected into the HPLC, whilst 150 mL OPA was added to 100 µL of the sample and 50 µL was injected into the HPLC.

## RESULTS AND DISCUSSION

**Pathogenicity**: The degree of pathogenicity was defined on the basis of non-emerged seedling (%) of the present investigations are shown in Table (2) and (3).All tested isolates of *F. verticilloides* affected the survival rate and vigour of seedlings. Two isolates (No.1 and No.2) appeared as high pathogenic by inducing (62.22 and 64.44%.) nonemerged seedlings, another three isolates (No.3, No.4 and No.9) classified as moderately (44.44, 55.55 and 51.11%) non-emerged seedlings. Isolates mentioned No.5, No.6 ,No.7, and No.8 as less pathogenic, recorded between (24.44%- 37.77%) non-emerged seedlings. Whereas, only one isolate (No.10) has very low pathogenic degree (13.33%) non emerged seedlings.

**Table 2: Degree of pathogenicity among ten *F. verticilloides* tested isolates.**

| Isolate No. | Isolate origin | Non-emerged seedlings % | Pathogenicity degree |
|---|---|---|---|
| 1 | Beheira | 62.22 | Highly pathogenic |
| 2 | Beheira | 64.44 | Highly pathogenic |
| 3 | Beheira | 44.44 | Moderatly pathogenic |
| 4 | Ismailia | 55.55 | Moderatly pathogenic |
| 5 | Ismailia | 37.77 | Low pathogenic |
| 6 | Ismailia | 26.66 | Low pathogenic |
| 7 | Kafr El-Sheikh | 33.33 | Low pathogenic |
| 8 | Garbia | 24.44 | Low pathogenic |
| 9 | Garbia | 51.11 | Moderatly pathogenic |
| 10 | Garbia | 13.33 | Very low pathogenic |
| control | | 2.22 | |

The obtained data show different degrees of pathogenicity among the tested isolates instead of being originated from the same locality. As in Garbia isolates which show various levels as moderate, low and very low (Table. 2).

The same results were revealed by determination of disease severity (%), whereas, isolates having high pathogenicity degree, recorded higher severity percentage more than the remaining tested isolates (49.8%, 51.6%), Low surviving healthy seedling (%) as (35.56 and 37.56%) and lowest plant vigour. The survival ratio of seedlings (Healthy) estimated from seed inoculation with different *F. verticilloides* tested isolates ranged from (35.56-86.67%) were developed from isolates (No.2 and No.10) respectively, which were generally lower than in the control (97.67%). The observed isolates reduced root length in comparison with the epicotyle growth rate. Isolates (No.3, No.4 and No.9) were moderatly pathogenic, based on their effect on root growth. However, isolate (No.6) expressed relative similar affects on root growth and classified according to non-emerged seedlings (%) and disease severity as a low pathogenic isolate (Table 3).

**Table 3: Effect of artificial inoculation by *F. verticilloides* tested isolates on maize seedlings.**

| Isolate No. | Plant vigour | | | | Healthy* seedlings % | Disease* severity % |
|---|---|---|---|---|---|---|
| | Length (cm) | | Dry weight (g) | | | |
| | Root | Epicotyl | Root | Epicotyl | | |
| 1 | 11.51 i | 8.88 j | 1.23 j | 0.39 j | 37.78 j | 49.8 b |
| 2 | 11.10 j | 12.56 h | 1.20 k | 0.37 k | 35.56 k | 51.6 a |
| 3 | 15.74 g | 13.61e | 1.38 h | 0.51 i | 55.56 g | 26.7 e |
| 4 | 15.66 g | 12.20i | 1.28 i | 0.54 h | 44.45 i | 33.3 c |
| 5 | 17.93 e | 13.33f | 2.63 b | 0.63 e | 62.23 f | 15.1 f |
| 6 | 16.74 f | 13.11g | 1.82 f | 0.61 f | 73.34 d | 10.7 h |
| 7 | 18.55 d | 14.01c | 1.99 e | 0.70 c | 66.67 e | 13.3 g |
| 8 | 18.64 c | 13.98d | 2.11 d | 0.83 d | 75.56 c | 9.8 i |
| 9 | 15.14 h | 12.13i | 1.41 g | 0.55 g | 48.89 h | 30.7 d |
| 10 | 22.49 b | 17.04b | 2.59 c | 0.66 d | 86.67 b | 2.7 j |
| Mean | 14.76 | 13.08 | 1.76 | 0.58 | 58.67 | 24.4 |
| Control | 33.07 a | 23.51a | 3.78 a | 1.11 a | 97.78 a | 0 k |
| LSD (0.05) | 0.101 | 0.166 | 0.01 | 1.57 | 1.28 | 0.005 |

*** Values of column followed by the same letters are not significantly different (P=0.05).**

The obtained results support the work of Desjardins *et al*., 1995, Munkvold and Carlton, 1997 and Krnjaja *et al*., 2007, where the latter study found that all tested isolates *F. verticilloides* originated from maize and wheat expressed different pathogenicity degrees to maize seedlings according to the reduction of seed germination(%), survival rate (%) and plants vigour . There was a tendency for isolates from different hosts to have similar values for pathogenicity, where these results are of practical importance in maize and wheat crop rotation and breeding for resistance towards this pathogen. Otherwise, Jardine and Leslie, 1999 reported that four strains of *F. verticilloides* were tested for aggressiveness towards two maize inbred lines

inducing significantly longer stalk lesions than in the control and they regarded as potential pathogens on mature maize plants.

Fumonisins belong to a mycotoxin group produced by *F. moniliforme* (syn. *F. verticilloides*) on maize, only FB1, FB2 and FB3 have been detected naturally in grains. Considering the worldwide contamination of maize by this pathogen in the field and that toxin is produced in the pre-drying step of harvested grains, showing positive correlation with damage kernels percentage and the toxin production (Ono *et al*., 2006)

The present results are consistent with the hypothesis that the host preferences described for *F. verticilloides* is related to its preferred host. Data in figure(1) show fumonisin B1 levels was detectable after 30 days of cultures, obtained by the tested isolates, whereas, those isolates observed more aggressiveness toward maize seedlings having higher ability in producing of the toxin. *i.e*. (No.1) and (No.2) Isolates were found to be highly pathogenic based on its pathogenicity and also by inducing higher percentages of disease severity. Both isolates expressed clear ability to synthesize relative high amounts of fumonisin B1 *in vitro* quantified as (1,500 and 1,680 µg/\mL). This results is in agreement with a previous study by Desjardins *et al.,* (1995) who found that aggressiveness, as assessed by maize seedlings blight, was correlated with the ability to produce high levels of fumonisin *in vitro*. In contrast, Jardine and Leslie, (1999) indicated that the aggressiveness of 4 tested strains of *F. verticilloides* towards mature maize plants for inducing stalk rot and the ability to produce fumonisins *in vitro* were not correlated.

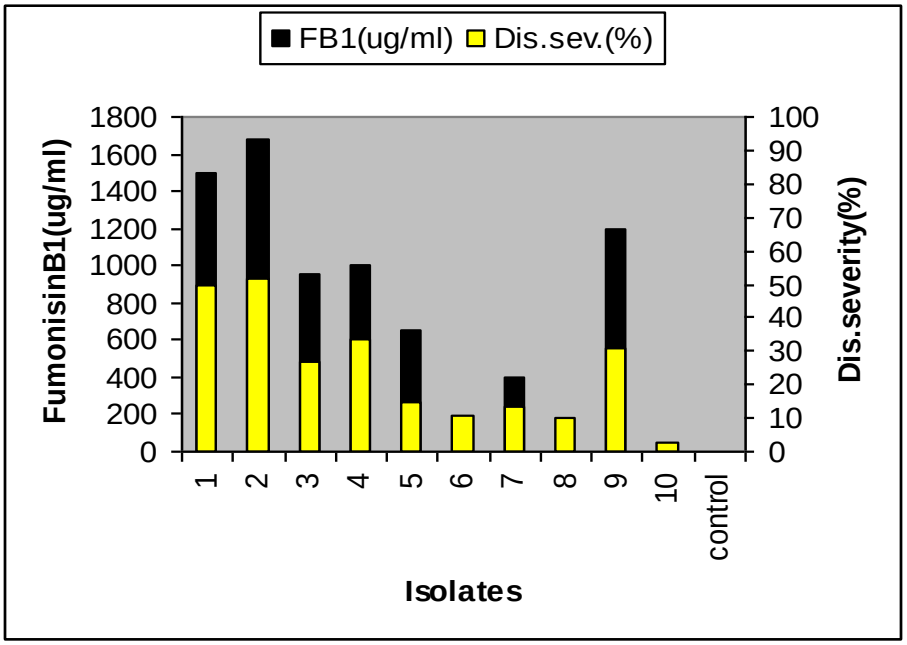


**Figure (1). Aggressiveness of *F.verticilloides* isolates related to Fumonisin B1 (µg/\mL) production.**

Similar trend in this study was recorded by various amounts of FB1 recovered by the remainder tested isolates, where the detectable quantities produced by each isolate directly proportional with its affinity to pathogenize maize seedlings (Figure.1).

## REFERENCES

Abbas, H.K., and Boyette, C.D. 1992. Phytotoxicity of fumonisin B1 on weed and crop species. Weed Technol. 6:548-552.

Bacon, C.W., Hinton, D.M., and Richardson, M.D. 1994. A corn seedling assay for resistance to *Fusarium moniliforme*. Plant Dis. 78:302-305.

Bailly, J.D., Querin, A., Tardieu, D., and Guerre, P. 2005. production and purification of fumonisins from a highly toxigenic *Fusarium verticilloides* strain. Revue Med. Vet. 156:547-554.

Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P., and Back house, D. 1994: Laboratory for *Fusarium* Research, Third edition, *Fusarium* research Laboratory, Department of crop sciences, University of Sydny and Royal Botanic Garden, Sydney, 133.

Chulz, S.N., Ramirez, M.L., Farnochi, M.C., Pascale, M., Visconti, A., and March, G. 1996. *Fusarium* and Fumonisin occurrence in Argentinian corn at different ear maturity stages. J. Agric. Food chem. 44:2797-2801.

Cvetnic, Z., Pepeljnjak, S., and Sevgic, M. 2005. Toxigenic potential of *Fusarium* species isolated from non-harvested maize, Arh Hig Rada Toksikol. 56:275-280.

Desjardins, A.E., Plattner, R.D., and Leslie, J.F. 1995. Genetic analysis of fumonisin production and virulence of *Gibberella fujikuroi* mating population A (*Fusarium moniliforme*) on maize (*zea mays*) seedlings. Appl. Environ. Microbiol. 61:79-86.

Dowell, F.E., Pearson, T.C., Maghirang, E.B., Xie, F. and wiklow, D.T. 2002. Reflectance and transmittance spectroscopy applied to detecting fumonisin in single corn kernels infected with *Fusarium verticilloides*. Cereal chemistry. 79:222-226.

Galvano, F., Pietri, A., Bertuzzi, T., Bognanno, M., Chies, L.,Angelis, A.and Galvano, M. (1997), Activated carbons: In vitro affinity for fumonisin B1 and relation of adsorption ability to physicochemical parameter- J. food Protection, 60.985-991.

Jajic, I.M., Bocarvo- Stancic, A. S., and Bijelic, M.B. 2007. Investigations of the capability of *Fusarium* isolates from corn for biosynthesis of fusariotoxins. Proc. Nat. Sci., Matica Srpska Novi Sad. 113:125-133.

Jardine, D.J., and Leslie, J.F. 1992. Agressiveness of *Gibberella fujikuroi* (*Fusarium moniliforme*) isolates to grain sorghum under greenhouse conditions. Plant Dis. 76:897-900.

Jardine, D.J., and Leslie, J.F. 1999. Agressiveness to mature maize plants of *Fusarium* strains differing in ability to produce fumonisin. Plant Dis. 83:690-693.

Krnjaja, V.S., Levic, J.T., Stankovic, S.Z. and Tomic, Z.M. 2007. Pathogenicity and diversity of vegetative compatibility of *Fusarium verticilloides*. Proc. Nat. Sci., Matica Srpska Novi Sad. 113:103-111.

Lamprecht, S.C., Marasas, W.F.O., Alberts, J.F., Cawood, M.E., Gelderblom, W.C.A., Shephard, G.S., Thiel, P.G., and Calitz, F.J. 1994. phytotoxicity of fumonisins and TA- toxin to corn and tomato. Phytopathology. 84:383-391.

Leslie, J.F., Plattner, R.D., Desjardins, A.E., and Klittich, C.J.R. 1992. Fumonisin B1 production by strains from different mating populations of *Gibberella Fujikuroi* (*Fusarium* section liseola). Phytopathology 82:341-345.

Maçka, M. 1989. Fusaria as pathogens of cereal seedlings, pp. 329-355, in chelkowski, J. (ed), Fusarium Mycotoxins, Taxonomy and pathogenicity, Elsevier, Amsterdam- Oxford- New York- Tokyo, 492.

Marasas, W.F.O, Kellerman, T.S., Gelderblom, W.C.A, coetzer, J.A.W., Thiel, P.G. and van derl ugt, J.J. 1988. Leukoencephalomalacia in a horse induced by fumonisin Bl isolated from *Fusarium moniliforme*. Onderstepoort J. Vet. Res. 55, 197-203.

Molot, P.M., Simone, J. 1967. Technique de contamination artificielle des semences de Mais pa les Fusarioses, Revie de Zoologie Agricole et Appliquee. 1-3:29-32.

Munkvold, G.P., Mc Gee, D.C., and Carlton, W.M. 1997. Importance of different pathways for maize internal infection by *Fusarium moniliforme*. Phytopathology 87:209-217.

Nelson, P.E., Plattner, R.D., Shackelford, D.D., and Desjardins, A.E. 1991. Production of fumonisins by *fusarium moniliforme* strains from various substractes and geographic areas. App. Environ. Microbiol. 57:2410-2412.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. *Fusarium* species. An illustrated manual for identification, The Pennsylvania State University press, University park and London, 193.

Ono, E.Y.S., Biazon, L., Da silva, M., Vizoni, E., sugiura, Y., Ueno, Y. and Hirooka, E.Y. 2006. Fumonisins in corn: correlation with *Fusarium* sp. count, damage kernels, protein and lipid content. Braz. Arch. Biol. technol. 49:1-11.

Schaafsma, A.W., Nicol, R.W., Savard, M.E. Sinha, R.C., Reid, L.M., and Rottinghaus, G. 1998. Analysis of *Fusarium* toxin in maize and wheat using thin layer chromatography. Mycopathologia. 142:107-113.

Shelby, R.A., white, D.G., and Bauske, M.E. 1994. Differential fumonisin production in maize hybrids. Plant Dis. 78:582-584.

Shephard, G.S., Van schalkwyk, D.J. and koch, K.R. 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei southern Africa. J. Agric. Food chem.. 38:1990-1903.

Sydenham,E.Y.,Shephard,G.S.,and Thiel,P.G.1992. Liquid chromatographic determination of fumonisins B1,B2 and B3 in foods and feeds .Journal AOAC International .75:313-317.

Thiel, P.G., Gelderblom, W.C.A., Marasas, W.F.O., Nelson, P.E., and Wilson, T.M. 1986 Natural occurrence of monilifomin and fusarin C in corn screenings known tobe heterocarcinogeic to rats. J. Agric. Food chem. 34:773-775.

U.S. FDA\CFSAN. 2001. Draft guidance for industry: Fumonisin levels in human food and animal feeds. http:\\www. Cf san. Fda. gov\dms\fumongu 2. html- US. FDA: Washington, DC.

Ueno, Y., Aoyama, S., Sugiura, Y., Wang, D.S., lee, U.S., Hirooka, E.Y., Hara, s., Karki, T. and chen, 1993. A Limited surveys of fumonisin corn and corn-based products in Asian countries. Mycotoxin Research. 9:27-34.

Wang, D.S., Liang, Y.X., Iijima, K., Sugiura, Y., Tanaka, T., chen, G., Yu, S.Z. and Ueno, Y. 1995. Co-contamination of mycotoxins in corn harvested in Haimen, a high risk area of primary liver cancer in china. Mycotoxins. 41:67-70.

Wicklow, D.T., weaver, D., and Throne, J. 1998. Fungal colonists of maize grain conditioned at constant temperatures and humidities. J. stored prod. Res. 34:355-361.

Yazdanpanah, H., Gheidari, P.E., Zarghi, A., and Mirkarimi, S.K. 2005. Survey of fumonisin B1 contamination of corn in Northern Iran during 2000. Iranian Journal of pharmaceutical Research. 4:221-225.

Yu, F.Y., and Chu, F.S. 1988. Analysis. Of fumomisin and AAL toxin by combination of HPLC and ELISA. JAOAC Intl. 81:749-756.

## دراسة القدرة الإمراضية لعزلات الفطر *Fusarium verticilloides* على الذرة الشامية و علاقتها بما تنتجه من fumonisin B1

**مني محمد سعيد محمد نور الدين**

**مركز البحوث الزراعية- معهد بحوث إمراض النباتات. الجيزة- مصر.**

تناول البحث إختبار القدرة الإمراضية لعشرة عزلات للفطر *Fusarium verticilloides* و المعزولة من حبوب الذرة الشامية المصابة بعفن الكيزان، و التي جمعت من عدة مناطق بمحافظات مصر. و تم تقييم هذه العزلات بإجراء اختبارات مختلفة، من حيث تأثرها على نسبة إنبات البذور و نسبة ظهور البادرات Seedlings emergence، تأثيرها على حيوية و قوة نمو البادرات Vigour index و كذلك النسبة المئوية لشدة المرض Disease severity، حيث أظهرت العزلات جميعها قدرات مختلفة على إحداث الإصابة للبادرات، و أدت العدوى بتلك العزلات المختبرة إلى انخفاض في نسب الإنبات للبذور و انخفاض في حيوية البادرات المصابة بتأثيرها السلبي على أطوالها و أوزانها مقارنة بالكنترول, و تفاوتت النسب المئوية لشدة المرض ما بين (2.7-51.6%) على التوالي .

اختبرت العلاقة بين قدرة تلك العزلات الفطرية على إحداث الإصابة لبادرات الذرة الشامية وقدرتها على إنتاج السم الفطري fumonisin B1 معمليا. فأعطت العزلات مؤشرا على إمكانية قدرتها على تخليق هذا السم على بيئات النمو، و تباينت قدرة بعض العزلات على إنتاج السم الفطري وتراوحت تركيزاتها ما بين (400-1.680ميكروجرام/ مل). حيث إظهرت النتائج علاقة ايجابية بين قدرة العزلات على إنتاج هذا السم الفطري و بين قدرتها على إحداث إصابة مرضية لبادرات الذرة الشامية.