
The toxicity of fumonisin B₁ in chicken embryos

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Cantaloupe (*Cucumis melo* L. var. *reticulatus* Ser.) is one of the most famous cucurbit crops in Egypt for local consumption and exportation. It is considered as an untraditional winter crop and became one of the most important exportation crop to the foreign markets. *Alternaria* spp. *Fusarium semitictum*, *F. subglutinans-1*, *F. solani*, *F. subglutinans-2* and *Rhizopus stolonifer* were isolated from naturally rotted fruits showing fruit-rot symptoms that collected from markets. Pathogenicity test using the isolated fungi *i.e.* *Alternaria* spp. *Fusarium semitictum*, *F. subglutinans-1*, *F. solani*, *F. subglutinans-2* and *Rhizopus stolonifer* revealed that, all isolated fungi were pathogenic and caused fruit rot disease on Galia cantaloupe cv. *Alternaria* sp. and *R. stolonifer* were weak parasites. The *Fusarium* spp. can infect all the tested hosts, *i.e.* cantaloupe cucumber, squash and melon, but with different values of disease severity. The ability of *F. semitictum* to produce Fumonisin (FB₁) that affected the mortality of chicken embryos indicated a nonlinear dose response relationship, eggs injected with 200µg FB₁. Microscopic examination of embryos that died during incubation period.

Keywords: Cantaloupe, Fruit-rot, *Fusarium* spp., Fumonisin B₁, Chicken embryo.

Introduction

Cantaloupe (*Cucumis melo* L. var. *reticulatus* Ser.) is one of the most famous cucurbit crops in Egypt for local consumption and exportation. It is considered as an untraditional winter crop and became one of the most important exportation crops to the foreign markets. The demand amounts from cantaloupe fruits for local consumption and exportation are annually increased. Cantaloupe plants are liable to infection by bacterial, fungal and viral diseases, in addition to nematode infection and physiological disorders (Agerteret *al.*, 2000; Helall, 2004, Muhanaa, 2006 and Ashour, 2009). However, fruit-rots are among the most destructive constrained for its production (Zitter, 1998 and Seebold, 2010).

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Mehl and Epstein (2007) reported that *Fusarium* spp. were collected from cucurbit fruits in production fields in the San Francisco Bay area, CA, from a produce distributor in San Francisco, and from grocery stores in Davis, CA and the San Francisco Bay Area.

Al-Sadiet *et al.* (2011) isolated *Fusarium equiseti* and *F. solani* diseased cucumber fruits. El-Mougy *et al.* (2012) isolated different root rot fungi i.e. *Alternaria solani*, *Fusarium solani*; *F. oxysporum*; *Rhizoctoniasolani*; *Sclerotium rolfsii*; *Macrophomin aphaseolina* and *Pythium* sp. were isolated from various vegetables, i.e. cucumber, cantaloupe, tomato and pepper grown in plastic houses under protected cultivation system and showing root rot and or damping-off disease symptoms.

Latiffah *et al.* (2013) reported that *Fusarium semitectum* is a widespread species occurring in various types of substrate. They added that fifteen *F. semitectum* isolates were recovered from several types of vegetable fruits showing fruit rot symptoms, namely long bean (*Vignasesquipedalis*), okra (*Abelmoschus esculentus*), loofa (*Luffa acutangula*), bitter melon (*Momordica charantia*), cucumber (*Cucumis sativus*) and green chilli (*Capsicum annuum*). The identification of the *F.* isolates was based on morphological characteristics of macroconidia and microconidia, presence of mesoconidia and colony pigmentation.

Al-sadi *et al.* (2011) reported that *Fusarium solani* and *Fusarium equiseti* were pathogenic on cucumber fruits, with *F. equiseti* being the most aggressive.

Abu Bakaret *et al.* (2013) found that all the tested *Fusarium* isolates were pathogenic on tomato with different severity levels. The non-inoculated controls showed no symptoms of fruit rot. The most virulent was *F. oxysporum* isolate B711T with DSI 93.75%, while the least were isolates of *F. solani* (B647T) and *F. oxysporum* (B727T) with DSI 37.5%. Majority of the isolated *Fusarium* species can potentially produce mycotoxins as their secondary metabolites. The potential production of mycotoxins by pathogenic isolates of *Fusarium* species in contaminated tomato fruits could pose health hazards when consumed.

Members of the genus *Fusarium* are often regarded as being soilborne, but they are also present in the air, water, and organic materials (Nelson *et al.*, 1981). The genus *Fusarium* is undoubtedly one of the most important groups of plant pathogenic fungi throughout the world, with *Fusarium oxysporum* .Schlecht. Erndend. Snyd.&Hans., being the most economically important member of this genus due to the existence of many pathogenic strains or forma especiales that affect a wide range of crops (Booth, 1971).

Fusarium mycotoxins are secondary metabolites of toxigenic *Fusarium* species which distribute ubiquitously in the world. There is increasing evidence

that these mycotoxins can provoke a broad spectrum of toxicities (Kubosaki *et al.*, 2008; Sudakin, 2003 and Voss *et al.*, 2002), and several specific animal mycotoxicosis such as equine leukoencephalomalacia (Wilson *et al.*, 1991) and porcine pulmonary edema (Haschek *et al.*, 2001) have been positively correlated with the exposure of certain *Fusarium* mycotoxins. Consumption of *Fusarium* mycotoxins-contaminated food stuffs has been implicated in human alimentary toxic aleukia, and is associated with the high incidence of human esophageal cancer in China and South Africa (Sun *et al.*, 2007; Rheeder *et al.*, 1992).

Fumonisin B₁ (FB₁) belongs to the recently (1988) discovered toxins fumonisins which are produced by *Fusarium verticilloides* (older synonym is *F. moniliforme*) and *F. proliferatum*, fungi that commonly contaminate maize. It has been also claimed that *F. napiforme*, *F. anthophilum*, *F. dlamini* and *F. nygamai* are able to produce FB₁ (EHC, 2000 and NTP, 1999).

Fumonisin B₁ (FB₁) has been found as natural contaminant in maize and maize based food from many parts of the world, e.g. the US, Canada, South Africa, Nepal, Australia, Thailand, The Philippines, Indonesia, Mexico, France, Italy, Poland, and the Mediterranean area (Spain, Italy and others) (Eriksen and Alexander, 1998; EHC, 2000 and Antonio *et al.*, 2003). The predominant member of the fumonisins is fumonisin B₁ (FB₁), it inhibits cell growth and causes accumulation of free sphingoid bases and alteration of lipid metabolism in *Saccharomyces cerevisiae*. FB₁ is phytotoxic, damages cell membranes and reduces chlorophyll synthesis. It also disrupts the biosynthesis of sphingolipids in plants and may play a role in the pathogenicity of maize by fumonisin-producing *Fusarium* species (WHOG, 2000).

Until now, twenty-eight fumonisins have been isolated and they can be divided in four series known as A, B, C and P. FB₁, FB₂ and FB₃ are the principal fumonisins analyzed as natural (Yazar and Omurtag, 2008). Contaminants of cereals toxins fumonisins which are produced by *Fusarium verticilloides* (older synonym is *F. moniliforme*) and *F. proliferatum*, fungi that commonly contaminate maize. It has been also claimed that *F. napiforme*, *F. anthophilum*, *F. dlamini* and *F. nygamai*, *F. subglutinans*, *F. solani*, *F. semitectum*, are able to produce fumonisins (EHC, 2000; NTP, 1999; Marasas, 2001 and Chibundu *et al.*, 2008).

The most common species of *Fusarium* were produced fumonisin F. *moniliforme* (60.7%) and *F. nygamai* (35.4%) followed by *F. semitectum*, *F. subglutinans*, *F. proliferatum*, *F. dlamini*, *F. solani*, *F. oxysporum* and *F. napiforme*. Reported by (Magnoli *et al.*, 1999).

Recent data on the epidemiology of the common mycotoxigenic species of *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium* in infected or colonized

plants, and in stored or processed plant products from the Mediterranean area are reviewed by (Antonio *et al.*, 2003). The Mediterranean basin is a large geographical region with a temperate climate supporting the cultivation of a wealth of field and green house crops with a high risk of mycotoxin contamination. The most important mycotoxins that occur in the Mediterranean basin are aflatoxins (B₁, B₂, G₁ and G₂) in dried fruits and nuts, ochratoxin A in grapes and raisins as well as trichothecenes and fumonisins in cereals (Tsitsigiannis *et al.*, 2012).

There for we studied the predominant member of the fumonisins toxins which fumonisin B₁ (FB₁), in Egyptian cantaloupe fruit rot samples that collected from markets and different areas of Giza (a government in Egypt which is a part of Mediterranean area). *Fusarium* was the genus isolated from the markets samples with *F.subglutinans*, *F.solani* and *F.semiticum*. Fumonisin B₁, isolated from culture material contaminated with *F. moniliforme*, was found to be hepatocarcinogenic and hepatotoxic to rats (Voss *et al.*, 1990 and Gelderblom *et al.*, 1991) and horses (Marasas *et al.*, 1988). Fumonisin B₁ was identified as the noninfectious causative agent of LEM in horses (Marasas *et al.*, 1988) and pulmonary edema in pigs (Harrison *et al.*, 1990 and Haschek *et al.*, 1992). In vitro studies by Norred *et al.*, (1992) indicated that FB₁ inhibits the biosynthesis of sphingolipids in hepatocytes and kills renal cells. Fumonisin B₁ is also toxic to chicken macrophages by causing a decrease in their functional activity (Qureshi and Hagler, 1992).

The present work was planned to study Isolation fungi responsible for causing cantaloupe fruit-rot. Pathogenicity test of isolated fungi. Extraction the toxin fumonisin (FB₁), from *Fusarium* spp. and its effect on chicken embryo in different concentration.

Materials and methods

Isolation, purification and identification of the causal organisms

Cantaloupe fruits showing different fruit-rot symptoms such as wet spots and dark brown as well as cracks in the outer shell of the fruit. were collected from different markets at Giza Governorate, Pieces of necrotic tissues on the fruits were taken off and surface sterilized in 2% sodium hypochlorite for 2 min. followed by several rinses in distilled water before being transferred onto potato dextrose agar medium (PDA). The grown cultures were picked up and purified by single spore method and/or hyphal tip technique. The purified cultures were maintained on PDA slants in a refrigerator at 5° C. Identification of the isolated fungi was carried out according to the cultural and micro

scopical characteristics using the description of Booth (1971); Nelson *et al.* (1983); Barnett and Hunter (1998); Dugan (2006).

Pathogenicity tests

Pathogenicity test of *Fusarium* spp. was carried out using Cantaloupe *Galia* cultivar in plastic boxes. Cantaloupe fruits were surface sterilized by immersing them in 1% sodium hypochlorite solution for 2 min, then washed several times with sterilized water. Three fruits were put in each box and maintained in moisture conditions. The fruits were crushed with sterilized scalpel in 5 cm² area and 10 mm disk of any of the tested fungi was put on each crushed area of fruit. PDA disks only were put on fruits crushed with sterilized scalpel in 5 cm² area and served as control treatment. The inoculated fruits were noticed daily until one week. The rotted fruits were examined and classified into the devised scale (0-5) as proposed by Townsend and Heuberger (1943), where; 0= No apparent symptoms of fruit- rot are seen, 1= The rotted portion ranged from .1- 10 % of the fruit size , 2= The rotted portion ranged from more than 10 - 25 % of the fruit size, 3= The rotted portion ranged from more than 25 - 50 % of the fruit size, 4= The rotted portion ranged from more than 50 – 75 of the fruit size % and 5= The rotted portion more than 75 % of the fruit size . Disease severity was calculated using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (n \times v)}{5 N} \times 100$$

Where,

n = Number of fruits in each category.

v = Numerical values of symptoms of each category.

N = Total number of the examined fruits.

5= Maximum number of numerical values of symptoms category.

The Toxicity of Fumonisin B₁ in chicken embryos

Fumonisin B₁ was produced and isolated according to Marasas (2001) using sterile, yellow corn as the substrate and *Fusarium semitectum* as the organism. Fumonisin B₁ standard was purchased from Sigma Chemical Co. (St. Louis, MO), and other chemicals were of the highest purity commercially available. The fumonisin B₁ was analyzed and determination by HPLC, according to Sydenham *et al.* (1991).

A stock solution of B₁ was made by dissolving 1mg FB₁/ml MeoH: H₂O (1:1) to give concentration 1mgFB₁/ ml, dissolving 500ug FB₁/ml MeoH : H₂O (1:1) to give concentration 500 ug FB₁/ml and dissolving 250ug FB₁/ml MeoH : H₂O (1:1) to give concentration 250 ug FB₁/ml. The HPLC method described by Shephard *et al.* (1998) was used for determination the different concentration of FB₁.

In experiment, the total volume of solvent used per egg was 200 µl. The safety measures recommended by WHO (1998) were taken when handling different concentrations of FB₁.

Broiler chicken hatching eggs were obtained from veterinary Department at National Research Center and used in the experiment. Thirty fertile eggs weighing between 60 and 75g (mean of 67.5g) were selected and incubated at 37 °C and at 40-60 % relative humidity before treatment with automatic rotation of the eggs at 4-h intervals. Embryonating eggs were assessed for viability by candling each egg after 72 h of incubation. Eggs with a clinically normal embryo and an air space in the normal location were selected for use in the experiments. The selected eggs were randomly divided into six treatment groups. First group (control group) eggs were incubated as they were received from the hatching egg source, second group (drilled group) eggs were drilled with the injection needle, no solution was injected, third group (solvent group) eggs were injected with 200ul MeoH: H₂O (1:1v/v), fourth, fifth and sixth groups (treated groups) were injected with FB₁ at doses of 50 µg FB₁/egg, 100 µgFB₁/egg and 200 µg FB₁/egg respectively. Immediately after injection, the injection site was sealed with a drop of nontoxic white glue. All eggs were labeled with a pencil and then returned to the incubator with the large end of the egg in an upward position all eggs incubated for 18 days. All eggs with a viable embryo on day 18 were opened and visually assessed, and final mortality was calculated. Eggs with dead embryos at each candling time were opened and visually inspected for the presence of gross abnormalities. Viable embryos were returned to the incubator on day 18. Hatchability was determined on day 22 of incubation. All chicks that hatched were weighed on day 22.

The obtained data were subjected to statistical analysis of variance (ANOVA) whenever needed, using the statistical analysis system Assistat (version 7.6). Mean of treatments were compared by Duncan's multiple range test at level of 0.05% (Rafter *et al.*, 2002).

Results

Isolation, purification and identification of the causal organisms

Isolation trials from naturally rotted fruits showing fruit-rot symptoms (Fig. 1) yielded many fungal isolates. The purified fungi were identified as *F. subglutinans*-1, *F. solani*, *F. subglutinans*-2, *Fusarium semitictum*, *Alternaria* spp. and *Rhizopus stolonifer*.



Fig. 1. Cantaloupe fruits showing natural infection by fruit-rots

Pathogenicity tests

Pathogenicity tests of the isolated fungi Table (1) and (Fig. 2) reveal that all the isolated fungi were pathogenic to Gallia cantaloupe cv., where all the inoculated fruits were infected (100% infection). However, both and *R. stolonifer* were weak parasites. In this respect, *F. semitictum* was the most pathogenic one, being 61.4 % disease severity (Fig. 3) followed by *F. solani* then *F. subglutinans*-1 and *F. subglutinans*-2, being 56.3, 52.6 and 43.0%, respectively. Meanwhile, *R. stolonifer* caused the lowest disease severity followed by *Alternaria* sp, being 10.0 and 24.3 %, respectively.

Table 1. Pathogenicity test of the isolated fungi on Galia cantaloupe cv

The tested fungi	% Infection	% Disease severity
<i>Alternaria</i> sp.	100	24.3 ^c
<i>F. semitictum</i>	100	61.4 ^a
<i>F. subglutinans</i> -1	100	52.6 ^c
<i>F. solani</i>	100	56.3 ^b
<i>F. subglutinans</i> -2	100	43.0 ^d
<i>R. stolonifer</i>	100	10.0 ^f
Control.	0.0	0.0 ^g

a-g Significant at a level of 1% of probability ($p < .01$)

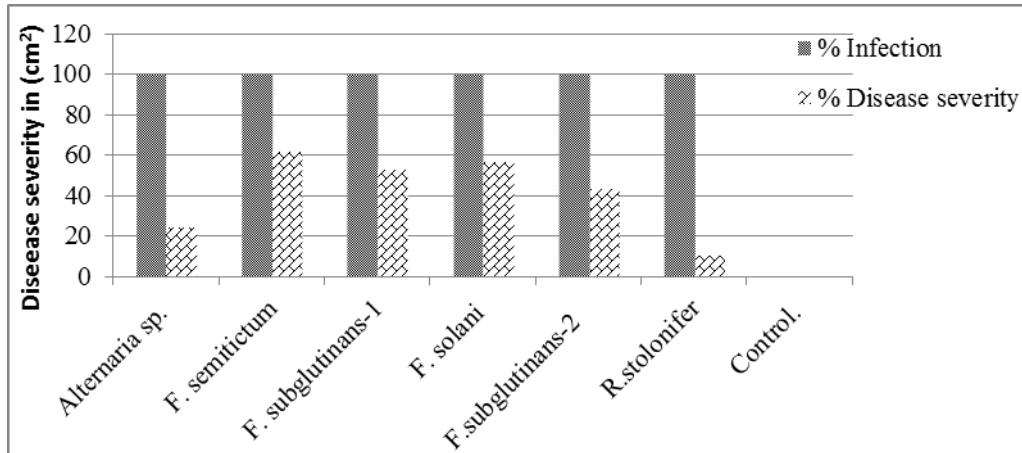
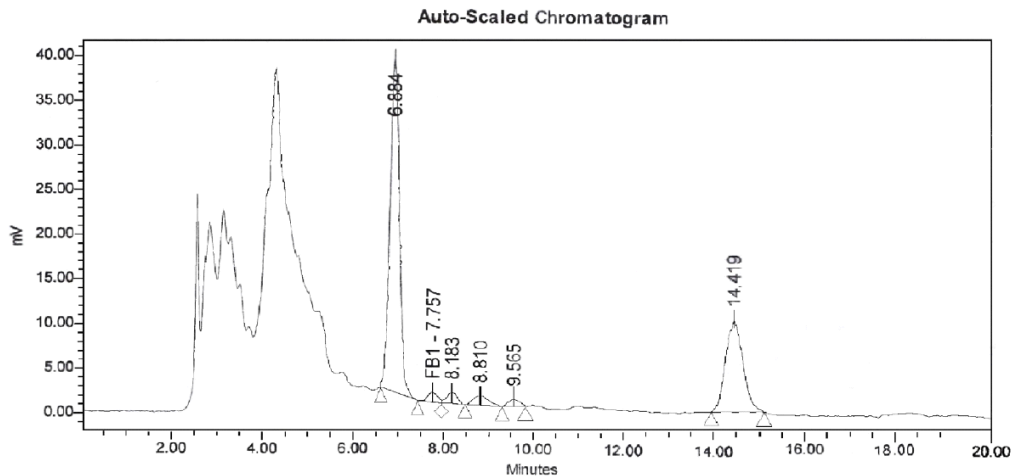


Fig. 2. Different effected of disease severity of fungi tested on Galia Cantaloupe cv.

The ability of different *Fusarium* isolates to produce fumonisin FB₁ was determined by grown the strains on liquid culture media Fig.(3)Fumonisin was the secondary metabolite mostly produced by the in vitro cultures of the *Fusarium* isolates analyzed.



F. semitectum

Fig. 3. HPLC of fumonisin production by *Fusarium semitectum* in culture media.

The highest mortality% of embryos within 21 days of incubation occurred at levels 100µg FB₁/egg and 200 µg FB₁/egg for groups (5 and 6) respectively were 100% for each. Mortality gradually increased throughout the 22-day incubation period Fig. (3).The effect of FB₁ on the mortality of chicken embryos indicated a nonlinear, dose response relationship Fig. (4). Embryonic

mortality ranged from 40 % in the control Egg to 100% in eggs injected with 200 μg FB_1 Table (2). Cumulative mortalities of embryos exposed to 50, 100 and 200 μg of FB_1 were significantly higher than those of the controls. Cumulative mortality of embryos exposed to FB_1 at 50 $\mu\text{g}/\text{egg}$ was not significantly different than control group solvent of the controls. When methanol :water was injected into the air space of eggs, the mortality of chicken embryo was 40% through 21 days of incubation, While it was 60% at 50 μg FB_1/egg (group 4).

Table 2. The response of embryonic mortality of chicken embryo exposed to fumonisin B₁ (FB_1)

	NO.OF injected	eggs	Days of incubation	of mortality at 21 day	of incubation
FB1 Injected μg				No. of death	%
control group	5		18	0\5	0 c
drilled group	5		18	0\5	0 c
solvent group	5		18	2\5	40 bc
group 4 (50 μg)	5		18	3\5	60 ab
group 5 (100 μg)	5		18	5\5	100 a
group 6 (200 μg)	5		18	5\5	100 a

^{a-c}Mortality values with different superscripts differ significantly ($p < 0.05$).

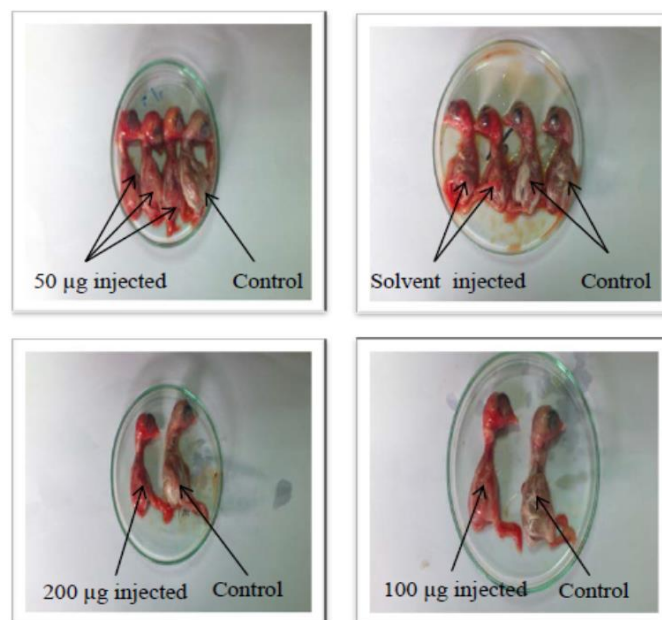


Fig. 3. Chicken embryos of dark red color and smaller in size in treated groups with FB_1 compared with control.

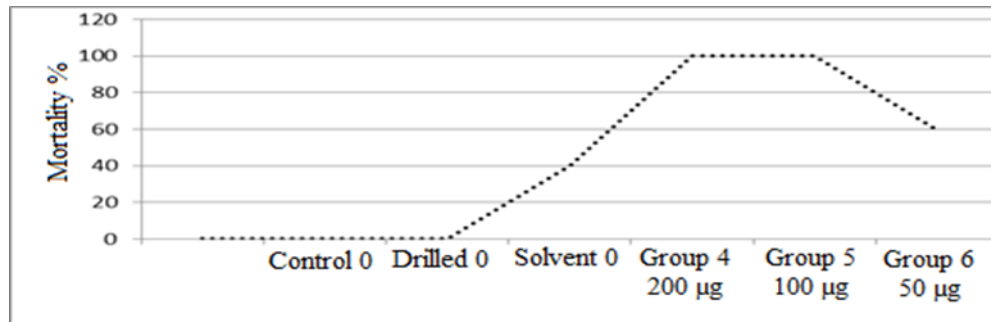


Fig. 4. The dose- response of mortality in chicken embryo exposed to fumonisinB₁ (FB₁) after 22 days of incubation.

Microscopic examination of embryos that died during incubation did not reveal any gross structural abnormalities of the head, beak, or limbs. However, all dead embryos taken from eggs on Day 18 had extensive hemorrhages in the head, neck, and thoracic.

There was no morphological difference between embryos from FB₁-exposed eggs and embryos from control eggs that survived 18 day of incubation, except the color there were dark reed in treated groups compared with control on which there color are pink. Hatchability of control eggs was not significantly different compared with the hatchability of eggs injected with FB₁. There were no significant differences in the weights of chicken from eggs injected with FB₁ and weights of chickens embryo from control eggs (Table 3).

Table 3. Chicken embryo weight in different concentration of fumonisin B₁ (FB₁) in 18 days incubation

FB ₁ Injected (µg)	NO.OF injected	eggs	Days of incubation	embryo weight in (gm.)
control group	5		18	10.296 a
drilled group	5		18	10.828 a
solvent group	5		18	10.71 a
group 4 (50µg)	5		18	10.668 a
group 5(100µg)	5		18	8.59 b
group 6 (200µg)	5		18	6.646 c

^{a-c}Weight embryo values with different superscripts differ significantly ($p < 0.05$).

Discussions

Cantaloupe fruits and plants are subjected to several diseases, during germination and growth, which attack the crop and reduce the produced fruits and their quality.

Isolation trial from the rotted fruits yielded many fungal isolates. The isolated fungi were purified and identified as *Alternaria* spp., *Fusarium semitictum*, *F. subglutinans*-1, *F. solani*, *F. subglutinans*-2 and *Rhizopus stolonifer*. Many investigators isolated these fungi from cantaloupe fruits at different localities. These results are in harmony with those reported by many researches (Elmer, 1996; Bruton *et al.*, 1998; Zhang and Bruton, 1999; Kwon *et al.*, 2009 and Latiffah *et al.*, 2013).

Pathogenicity test of the isolated fungi from fruits revealed that all the isolated fungi were pathogenic and caused fruit rot disease on *Galia cantaloupe* cv. In this suspect, the highest percentages of fruit-rots severity were recorded by the four species of genus *Fusarium*, i.e. *Fusarium semitictum*, *F. subglutinans*-1, *F. solani* and *F. subglutinans*-2. The obtained results are in agreement with those obtained by Bruton *et al.* (1998); Zhang *et al.* (1999); Seebold (2010) and Latiffah *et al.* (2013).

The biological experiment shows that the administration of a single air sac injection of mycotoxin FB₁ into chicken eggs after 18 day of incubation was toxic to embryos. Bilayer, which, if impaired, may result in increased capillary permeability and rupture of red blood cells. This experiment shows that the fumonisin B₁ is toxic to chicken embryos and have no developmental effects on embryos that survive after exposure. This investigation also reveals that the toxicities of FB₁ being the most toxic. The toxicity of the fumonisins FB₁ in chicken embryos also indicates that the toxicity observed in animal experiments, in which *F. semitictum* culture material with a known level of FB₁ was incorporated into the diet, cannot be attributed only to the action of FB₁. These results further amplify the fact that the fumonisin is toxic compounds and should be considered an animal and human health hazard. Magnoli *et al.* (1999) reported that the most common species of *Fusarium* produced Fumonisin are *F. moniliforme*, *F. nygamai*, *F. semitictum*, *F. subglutinans*, *F. proliferatum*, *F. dlamini*, *F. solani*, *F. oxysporum* and *F. napiforme*.

The chicken embryo is a well-documented system to test the toxic effect of fumonisin FB₁ on embryonic development (Zacharias *et al.*, 1996). The chick embryo assay has been used as a preliminary screen to assess the toxic and teratogenicity responses from mycotoxins and other toxic chemicals (Henry and Wyatt, 2001). This experiment shows that the administration of a single air sac injection of this mycotoxin into chicken eggs after 18 day of incubation was toxic to embryos.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- Abu Bakar, A.I., Nur Ain Izzatizzati, M.Z. and UmiKalsom, Y. (2013). Diversity of *Fusarium* Species associated with post-harvest fruit rot disease of tomato. *Sains Malaysiana*, 42(7):911–920.
- Agerter, B.J., Gordon, T.R. and R.M. Davis (2000). Occurrence and Pathogenicity offungi associated with melon root-rot and vine decline in cantaloupe. *Plant Dis*, 84:224-230.
- Al-Sadi, A.M., Al-Said, F.A., Al-Kaabi, S.A., Al-Quraini, S.M., Al-Mazroui, S.S., Al-Mahmooli, I.H. and Deadman, M.L. (2011). Occurrence, characterization and management of fruit rot of immature cucumbers under greenhouse conditions in Oman. *Phytopathol.Mediterr* 50:421–429.
- Antonio, L., Antonio, B., Giuseppina, M., Antonio, M. and Giancarlo, P. (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops *European Journal of Plant Pathology* 109:645–667.
- Ashour, A.M.A. (2009). Effect of application of some systemic fungicides and resistance inducing chemicals on management of cantaloupe powdery mildew disease. *Egypt. J. Phytopathol* 37(1):1-8.
- Barnett, H.L. and Hunter, B.B. (1998). *Illustrated Genera of Imperfect Fungi*. 4th ed. APS Press, St. Paul, Minnesota. pp. 218.
- Booth, C. (1971). *The genus Fusarium*. Commonwealth Mycological Institute, Key, Surrey, England. UK, pp. 237.
- Bruton, B.D., J.X. Zhang, Howell, C.R. and Miller, M.E. (1998). *Fusarium* species causing cantaloupe fruit rot in the lower riogrande valley of Texas. *South Texas Melon Committee Annual, Research Report, Experiment Station*, pp. 17-24.
- Chibundu, N.; Odeode, C. and Stepheno, F. (2008). Zearalenone Production by Naturally Occurring *Fusarium* Species on Maize, Wheat and Soybeans from Nigeria *J. Biol. ENVIRON. SCI.*, 6:77-82.
- Dugan, F.M. (2006). *The Identification of Fungi: An Illustrated Introduction With Keys Glossary and Guide to Literature*. APS. Press, St. Paul, Minnesota, USA. pp.176.
- EHC, (2000). *Environmental Health Criteria 219: fumonisin B₁*, International Programme on Chemical Safety (IPCS; UNEP, ILO and WHO). Eds. W.H.O. Marasas, J.D. Miller, Riley, R.T. and A. Visconti. WHO, Geneva, pp. 150.
- Elmar, W.H. (1996). *Fusarium* fruit rot of pumpkin in Connecticut. *Plant Dis*, 80:131-135.
- El-Mougy, N.S; Abdel-Kader, M.M; Aly, M.D.E. and Lashin, S.M. (2012). Application of fungicides alternatives as seed treatment for Controlling root rot of some vegetables in Pot Experiments. *Advances in Life Sciences* 2(3):57-64.
- Eriksen, G. and Alexander, J. (1998). *Fusarium* toxins in cereals – risk assessment. *Tema Nord* 1998:502, Nordic Council of Ministers, Copenhagen, ISBN 92-893- 0149-X, 3 – 115.n to human esophageal cancer in Transkei. *Phytopathology* 82:353–357.
- Gelderblom, W.C.A., Kriek, N.P.J., Marasas, W.F.O. and Thiel, P.G. (1991). Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite fumonisin B₁ in rats. *Carcinogenesis* 12:1247–1251.
- Harrison, L.F., Colvin, B.M., Greene, J.T., Newman, L.E. and Cole Jr, J.R. (1990). Pulmonary edema and hydrothorax in swine produced by fumonisin B₁, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* 2:217–221.
- Haschek, W.M., Gumprecht, L.A., Smith, G., Tumbleson, M.E. and Constable, P.D. (2001). Fumonisin toxicosis in swine: an overview of porcine pulmonary edema and current perspectives. *Environmental Health Perspectives* 109:251–257.

- Haschek, W.M., Motelin, G., Ness, D.K., Harlin, K.S., Hall, W.F., Vesonder, R.F., Peterson, R.E. and Beasley, V. (1992). Characterization of fumonisin toxicity in orally and intravenously dosed swine. *Mycopathologia* 117:83–96.
- Henry, M.H. and Wyatt, R.D (2001). The Toxicity of Fumonisin B₁, B₂, and B₃, Individually and in Combination, in Chicken Embryos¹. *Poultry Science* 80:401–407.
- Hilall, Mervat R. (2004). Induced acquired resistance to cantaloupe powdery mildew by some chemicals under greenhouse conditions. *Egypt. J. Appl. Sci*, 19(1):82-90.
- Kubosaki, A., Aihara, M., Park, B.J., Sugiura, Y., Shibutani, M., Hirose, M., Suzuki, Y., Takatori, K. and Sugita-Konishi, Y. (2008). Immunotoxicity of nivalenol after sub chronic dietary exposure to rats. *Food and Chemical Toxicology* 46:253–258.
- Kwon, J.H., Chi, T.T.P. and Park, Chang-Seuk (2009). Occurrence of fruit rot of melon caused by *Sclerotium rolfsii* in Korea. *The Korean Society of Mycol.*, 37(2):158-159.
- Latiffah, Z., Nurul Huda, M.S. and Tengku Ahmad Akram, T.M.A. (2013). Characterization of *Fusarium semitectum* from isolates vegetable fruits. *Sains Malaysiana* 42(5):629–633.
- Magnoli, C.E., Saenz, M.A., Chiacchiera, S.M., Dalcerro, A.M. (1999). Natural occurrence of *Fusarium* species and fumonisin-production by toxigenic strains isolated from poultry feeds in Argentina. *Mycopathologia* 145:35-41.
- Marasas, W.F.O. (2001). Discovery and occurrence of the fumonisins: a historical perspective. *Environ. Health Perspect* 109:239-243.
- Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A., Coetzer, J.A.W., Thiel, P.G. and van der Lugt, J.J. (1988). Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res.* 55:197–203.
- Mehl, H.L. and Epstein, L. (2007). Wage and community shower drains and environmental reservoirs of *Fusarium solani* species complex group 1, a human and plant pathogen. *Environmental Microbiology*, doi:10.1111/j.1462-2920.01446.x.
- Muhanna, Naglaa A.S. (2006). Pathological studies on root-rot and vine decline of cantaloupe in Egypt. Ph.D. Thesis, Fac. Agric, Cairo Univ. pp. 218.
- Nelson, P.E., Horst R.K. and Woltz, S.S. (1981). *Fusarium* diseases of ornamental plants. In: Nelson TA and Cook RJ (eds), *Fusarium: diseases, biology and taxonomy*. Pennsylvania State University Press, University Park, Pennsylvania, pp. 121-128.
- 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, pp. 193.
- Norred, W.P., Wang, E., Hwangsoo, Y., Riley, R.T. and Merrill, A.H. Jr. (1992). In vitro toxicology of fumonisins and the mechanistic implications. *Mycopathologia* 117:73–78.
- NTP (1999). NTP technical report on the Toxicology and Carcinogenesis studies of fumonisin B1 (CAS NO. 116355-83-0) in F344/N rats and B6C3F1 mice (Feed studies). NTP TR 496, NIH Publication No. 99-3955, US Department of Health and Human Services, Public Health Service, National Institute of Health. pp. 1–46.
- Qureshi, M.A. and Hagler, W.M. (1992). Effect of fumonisin B₁ exposure on chicken macrophage function in vitro. *Poultry Sci.* 71:104–112.
- Rafter, J.A., Abell, M.L. and Braselton, J.P. (2002). Multiple comparison methods for means. *Society for Industrial and App. Math*, 44(2):259-278.
- Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S. and van Schalkwyk, D.J. (1992). *Fusarium moniliforme* and fumonisins in maize in relation to human esophageal cancer in Transkei. *Phytopathology* 82:353–357.

- Seebold, K. (2010). Fruit Rots of Cucurbits. Extension Plant Pathologist, Home Vegetable Gardening in Kentucky, ID-128 (University of Kentucky) <http://www.ca.uky.edu/agc/pubs/id/id128/id128.pdf>.
- Shephard, G.S. (1998). Chromatographic determination of the fumonisin mycotoxins, J. Chromatogr., 815:31-39.
- Sudakin, D.L. (2003). Trichothecenes in the environment: relevance to human health. Toxicology Letters 143:97-107.
- Sun, G., Wang, S., Hu, X., Su, J., Huang, T., Yu, J., Tang, L., Gao, W. and Wang, J.S. (2007). Fumonisin B1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. Food Additives and Contaminants 24:181-185.
- Sydenham, E.W., Shephard, G.S., Thiel, P.G., Marasas, W.F. and Stockenstrom, S. (1991). Fumonisin contamination of commercial corn-based human food stuffs. J. Agric. Food Chem. 39(11):2014-2018.
- Towsend, G.K. and Heuberger, T.W. (1943). Methods for estimating losses caused by diseases in fungicide experiments. Plant Dis., 2:340-343.
- Tsitsigiannis, D., Myrto, D., Polymnia, P. and Eleftherios, C. (2012). Biological control strategies of mycotoxigenic fungi and associated mycotoxins in Mediterranean basin crops Phytopathologia Mediterranea .51(1):158-174.
- Voss, K.A., Howard, P.C., Riley, R.T., Sharma, R.P. Bucci, T.J. and Lorentzen, R.J. (2002). Carcinogenicity and mechanism of action of fumonisin B₁: a mycotoxin produced by *Fusarium moniliforme* (=F. Verticillioides). Cancer Detection and Prevention 26:1-9.
- Voss, K.A., Plattner, R.D., Bacon, C.W. and Norred, W.P. (1990). Comparative studies of hepatotoxicity and fumonisin B1 and B2 content of water and chloroform/methanol extracts of *Fusarium moniliforme* strain MRC 826 culture material. Mycopathologia 112:81-92.
- WHO, (1998). Surveillance Programme for Control of Food-borne Infections and Intoxications in Europe. 7th Report. http://www.bgvv.de/internet/7threport/7threp_etryreps_fr.htm.
- WHOG (World Health Organization Geneva). (2000). Environmental Health Criteria 219,3.
- Wilson, T.M., Ross, P.F. and Nelson, P.E. (1991). Fumonisin mycotoxins and equine leukoencephalomalacia. Journal of the American Veterinary Medical Association 198: 1104-1105.
- Yazar, S. and Omurtug, G. (2008). Fumonisin, Trichothecene and zearalenone in Cereals. Int. J. Mol. Sci 9:2062-2090.
- Zacharias, C., Echten-Deckert, G.V., Wang, E., Merrill, A.H. and Sandhoff, K. (1996). The effect of fumonisin B1 on developing chick embryos: correlation between de novo sphingolipid biosynthesis and gross morphological changes. Glycoconjugate J. 13:167-175.
- Zhang, J.X., Bruton, B.D., Miller, M.E. and Isakeit, T. (1999). Relationship of developmental stage of cantaloupe fruit to black rot susceptibility and enzyme production by *Didymellabryoniae*. Plant Dis, 83:1025-1032.
- Zitter, T.A (1998). Fusarium Diseases of Cucurbits. IPM, Fact Sheet pp. 733.

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