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Effects of Some Chemical Compounds on the Growth of Fusarium Species

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Abstract
Fusarium species was isolated from soil of the Basra Province, South of Iraq, and cultured at 27C° for 5 days on three media were (Czapek Dox agar, CDA, malt extract agar, MEA, and potato dextrose agar, PDA). These media appeared different coonies of this fungus were whitish gray with pink center, white with concavely center, and white possessed pink center, respectively. In addition, no exudates were produced. Using lactophenol cotton blue stain, conidia were crescent with pointed ends and some of them are septate. Different chemical compounds were tested on a growth of the *Fusarium* species revealed a highest growth was noticed in potato dextrose broth (PDB) amended with aspartic acid followed by potassium chloride, magnesium sulfate and magnesium chloride. In addition, no growth in a presence of the ammonium acetate, potassium acetate, zinc chloride and zinc sulfate.
Keywords: *Fusarium* species, Growth curve, Aspartic acid

الملخص
تضمنت هذه الدراسة فطر الـ Fusarium المعزول من تربة محافظة البصرة في جنوب العراق وفيه ان الفطر كان قد ابدى نموا في درجة حرارة 27 م° على ثلاثة اوساط زرعية (اكار الزابكس، اكار مستخلص الشعير، واكار دكستروز البطاطا) لمدة 5 أيام. اظهر نمو الفطر مستعمرات مختلفة الالوان اذ ان نموه على اكار الزابكس شوهد بمستعمرات رمادية مبيضة بينما كانت بيضاء ذات وسط مقعر على اكار مستخلص الشعير وبيضاء ايضا على اكار دكستروز البطاطا لكنها غير مقعرة. اما لون مركز المستعمرات على جميع الاوساط الزرعية كان قد لوحظ باللون الوردي. الفحص المجهري كشف وجود ابواغ هلالية الشكل مدببة النهايات وبعض الابواغ كان مقسما. بالنسبة لتأثير بعض المركبات على نمو فطر الـ Fusarium فقد وجد ان أفضل نمو له ظهر في مرق دكستروز البطاطا المدعوم بحامض الاسبارتك تلاه كلوريد البوتاسيوم ثم سولفات المغنيسيوم واخيرا كلوريد المغنسيوم. لكن نمو الفطر في المرق انف الذكر الممزوج وبشكل منفصل مع اسيتات الامونيوم، اسيتات البوتاسيوم، كلوريد الزنك وسولفات الزنك فلم يظهر نموا بالمقارنة مع نمو الفطر في مرق دسنورز البطاطا لوحده.
الكلمات المفتاحية: فطر الـفوساريوم، منحنى النمو، حامض الاسبارتك

Introduction

Fusarium species are distributed in the soil as saprophytic fungi, and weak plant pathogens for several fruits and crops in subtropical, tropical, and temperate climate countries.[1, 2] This fungal species produce many secondary metabolites such as antimicrobial agents, trichothecens, equisetine, fusarenone etc.[3] There are different factors affecting fungal growth, for examples, temperature, pH, substrate and chemical elements.[4] Many chemical elements such as magnesium, potassium and phosphorus are required for the fungal growth. These elements are called macroelements which are supplied as salts for fungi e.g., magnesium sulfate and potassium phosphate as growth factors. Copper, zinc, calcium, iron, manganese and molybdenum as microelements are needed in very small quantities for growth of fungi as cofactors for fungal enzymes and functional proteins.[5]
Fungi also need vitamins in a very little amounts such as thiamine for growth. Some fungi are unable to utilize a source of the nitrate or ammonium.[6, 7] Therefore, nutritional effects have been studied in growth of fungi. Supply of amino acid or ammonium into nitrogen-starved cells and supporting them lead to activate these cells.[8] Based on the mentioned words, this study aimed to evaluate the effects of some chemical compounds on the growth of *Fussarium* species which was isolated from the soil.

Materials and methods

Identification of *Fusarium* species

Fusarium species was isolated from soil and obtained from the Mycological Research Laboratory, Education College for Pure Sciences, Basra University. This fungus was grown on three media were Czapek Dox agar (CDA), malt extract agar (MEA), and potato dextrose agar (PDA) at 27°C for 5 days. This fungus was morphologically identified according to prevous studies.[9, 10]

Effect of chemical compounds on growth of *Fusarium* species

Twelve of the chemical compounds were tested (Table 1) that 1 g of each compound was dissolved in 100 ml of potato dextrose broth (PDB) for getting a mixture which had pH was 5. Five ml of each mixture were placed in a screw glass tube and sterilized by autoclave at 121°C for 15 min. except PDB with thiamin-HCl or aspartic acid were sterilized by Millipore filter paper (0.2 µm). Plate contained 100 wells was used and 13 wells were selected. Aseptically, each well was loaded with 200 µl of the each mixture and wells contained PDB only were used as control. Cuvette was filled with 1 ml of PDB containing fungal cells and placed in O.D

device to estimate optical density of fungal cells that the final O. D was 0.01. All wells were inoculated with 100 µL of the fungal growth (0.01 O.D) and placed in bio-screening device (Fig.1) based on the following program: Temperature 28°C., incubation period was 7 days, shacking continuous**,** filtering 7:600 nm and Recording data at each hour. Final O.D was estimated to detect the effect of chemical compounds on the fungal growth.

Results and discusion

Fusarium species appeared different colony shapes at $27C^0$ on three various media. Colonies on CDA were whitish gray with pink center while reversible was gray with yellowish pink center. On MEA, the colonies were white with concavely center and reversible was yellow especially in center was dark. Contextually, PDA resulted in white colonies but the center and edges were pink besides yellow reversible especially in center. No exudates were produced from this fungus on all media. Colonies appeared in shape of wool or feathers on CDA, MEA (it possessed excellent growth) and PDA, the growth of an aerial mycelia was observed on the agar surface. Microscopically, lactophenol stained-conidia revealed crescent shape with pointed ends and some of them are septate (Fig. 1)

Effect of chemical compounds on growth of *Fusarium* species

Among twelve chemical compounds, the growth response of *Fusarium* speceis was better in a medium (PDB) amended with aspartic acid, and was followed by potassium chloride, magnesium sulfate and magnesium chloride. (Figures: 3, 4, 5, 6). While the growth response was obtained in PDB amended with KH_2PO_4 followed by Na_2HPO_4, thiamine-HCl and ammonium chloride was not better than PDB only (Figures: 7, 8, 9, 10). In addition that, no growth in a presence of the ammonium acetate, potassium acetate, zinc chloride and zinc sulfate (Figures: 11, 12, 13, 14).

The genus *Fusarium* is widely distributed over the world and considered as weak plant pathogen.[11, 12] The identification mostly depends on the morphology.[13] Similarly to other organisms, fungal growth is affected by environmental factors such as macroelements (carbon, nitrogen, phosphate, sulfate, potassium, magnesium, calcium…etc).In addition, growth of fungi needs to vitamins, amino acids but in very little mounts as microelements including calcium, copper, zinc, manganese and molybdenum.[13, 14, 15] Metals and vitamins are participants as growth factors and coenzymes lead to positively change in fungal enzyme. Vitamins and metals like Mg^{+2}, Zn^{+2}, Mn^{+2}, K^{+} have influence as activators by formation of enzyme-metal (E-M) complex which leads to increase the enzymatic activity.[16]

Majority of fungi have ability to utilize amino acids as source for carbon and nitrogen. Also many fungi require vitamins for example thiamine in very small amounts as carbon source.[17] Nutritional effects have been studied in the fungal growth. Supply of amino acid or ammonium into nitrogen-starved cells, and supporting phosphate for phosphate-starved cells in presence of glucose lead to activate PKA pathway without mediation by cAMP as second messenger. In the activation of PKA pathway, amino acids, ammonium and phosphate were sensed by transporter-receptor protein named as transceptors. Gap1for amino acid, and MeP2, and Ph084 for sensed ammonium and phosphate, respectively.[13] However, some fungi have no ability to utilize nitrate or ammonium as nitrogen source, therefore, they need to a single amino acid.[7-9] Many amino acids have an effect on growth of some *Fusarium* species. Aspartic acid led to increase the fungal biomass as well as high production of antimicrobial secondary metabolite.[1, 3] The effect of aspartic acid is stimulator to produce many secondary metabolites (aurofusarin, butenolide, diacetoxyscripenol, dipicolinic acid, fumonisin B , gentisyl alcohol, moniliformin, neosaloniol) from *F. saccahri* var.*elongatum*.[4] This study aimed to the assay separately the amended different substances including aspartic acid with PDB to show their effects on *Fusarium* species to be future works for production of secondary metabolites from the fungus for biotechnology and chemotaxonomy. The results of this study showed different growth curves of *Fusarium* species in PDB amended with one of different metals, thiamine-HCL and aspartic acid. It appeared that aspartic acid plays a major growth factor for *Fusarium* species.

There are many effects of amino acids on growth of *Fusarium* spp. Aspartic acid was first effective factor than other tested amino acids whereas it leads to increase the fungal biomass. pH factor affects in amount and speed of growth for microorganisms. Because each organism has certain pH in which grows, pH must be adjusted and controlled before the fungus is cultured. pH factor was studied and gave highest growth for *Fusarium* species at pH 8, and maximum antimicrobial secondary metabolite at pH 5. pH is an important factor was reported to obtain higher fungal growth and maximum amount of secondary metabolites included antimicrobial compounds.[9-11]

Conclusions

Fusarium species is can be isolated from soil of Basra Province, South of Iraq, and its growth is affected by addition of chemical compounds; therefore, PDA amended with aspartic acid is better medium for the isolation compared with PDA only. In addition, a growth stationary phase of this fungal species was remarkably noticed when the fungal growth was evaluated using various chemical substances amended with PDB.

Acknowledgments

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References

1. Ahmad M. S., AL-Nawawi M. A. Industrial Mycology. First. Ed. Arabic Aldar for Publication, Egypt. (1999), 59-70.

2. Carlile M. J., Watkinson S. C., Gooday, G. W. The Fungi. 2nd.ed. Academic Press, UK. (2001), 145-159.

3. Deacon J. W. Fungal Biology. 4th.ed. Blackwell Publishing, USA. (2006), 111.

4. Edel-Hermann V., Gautheron N., Mounier A., Steinberg C. *Fusarium* diversity in soil using a specific molecular approach and a cultural approach. *Journal of Microbiological Methods*. (2015), 111, 64-71.

5. Keller S. E., Sullivan T. M., Chirtel S. Factors affecting the growth of *Fusarium proliferatum* and the production of fumonisin B_1: oxygen and pH. *Journal of Industrial Microbiology and Biotechnology*. (1997), 19(*4*), 305-309.

6. Kistler H.C. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. *Phytopathology*. (1997), 87, 474 - 479.

7. Klittich C. R., Leslie J. F., Nilson P. E., Marasas W. O. *Fusarium thapsinum* (*Gebberella thapsina*): a new species in section Liseola from Sorgum. *Mycologia*. (1997), 89, 643-652.

8. Kodo N., Nakamura C., Kato H., Yoshizawa T., Mori N., Kaneda C. Restriction fragment length polymorphism of mitochondrial DNAs from seven *Fusarium* species causing *Fusarium* head blight. *The Japanese Journal of Genetic*. (1995), 70 (*3*), 437-451.

9. Nomila J., Merlin I. V. S., Christhundas N., Kumar P. P., Agastian P. Optimization of growth and bioactive metabolite production: *Fusarium solani*. *Asian Journal of Pharmaceutical and Clinical Research*. (2013), 6(*3*), 98-103.

10. Mwaniki P. K., Abang M. M., Wagara I. N., Wolukau J. N., Schroer H. J. Morphology, pathogenicity and molecular identification of *Fusarium* spp. from wilting eggplant in Tanzania. *African Crop Science Conference Proceedings.* (2011), 10, 217-221.

11. Satyanarayana U., Chakrapani U. Biochemistry. Third ed. Elsevier, India Pvt. Ltd (2006), 85-634.

12. Singh V., Haque S., Niwas R., Srivastava A., Pasupuleti M. and Tripathi, C. Strategies for fermentation medium optimization: An in-depth review. *Frontier Microbiololgy*. (2017), 7, 1-16.

13. Stepien L., Gromadzka K., Chlekowski, J. Polymorphism of mycotoxin biosynthesis genes among *Fusarium equiseti* isolates from Italy and Poland. *Journal of Applied Genetic*. (2012), 53 (*2*), 227-236.

14. Tayunk K., Barik B. P., Jha D. K., Deka D. C. Identification and characterization of antimicrobial metabolite from an entophytic Fungus, *Fusarium solani* isolated from bark of Himalayan Yew. *Mycosphere*. (2011), 2(*3*), 203-213.

15. Thevelein J., Bonini B., Castermans D., Haesendonckx S., *et al.,* Molecular genetics and physiology of nutrient regulation in yeast. In physiology of yeast and filamentous fungi (PYFF3). 3rd.ed. European Federation of Biotechnology Conference. (eds. Kuokka-Ihalainen, A.; Saloheimo, M. and Pakula,T) (2007), 25.

16. Zain M. E., Bahkali A. H., Al-Othman M. R., Khalil A. M. Advantage of using secondary metabolites in fungal chemotaxonomy. *Australian Journal of Basic and Applied Sciences*. (2012), 6 (*13*), 95-103.

17. Zain M. E., Bahkali A. H., Al-Othman M. Effect of chemical compounds on amino acid content of some Fusarium species and its significance to fungal chemotaxonomy. Journal of Saudi Chemical Society. (2012), 16 (2), 183-192.

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Table 1: Chemical compounds were used for growth of the *Fusarium* species.

Chemicals	Manufacture (Company)
Ammonium acetate	Analar, England
Aspartic acid	Sigma, USA
Ammonium chloride	Fison, England
KH_2Po_4	Fisher, UK
Na_2HPo_4	Fison, England
Magnesium chloride	Fisher, UK
Magnesium sulfate	Fisher, UK
Potassium acetate	Sigma, USA
Potassium chloride	Melford, UK
Thiamin-HCL	Duchefa Biochemie
Zinc chloride	Fison, England
Zinc sulfate	Sigma, USA

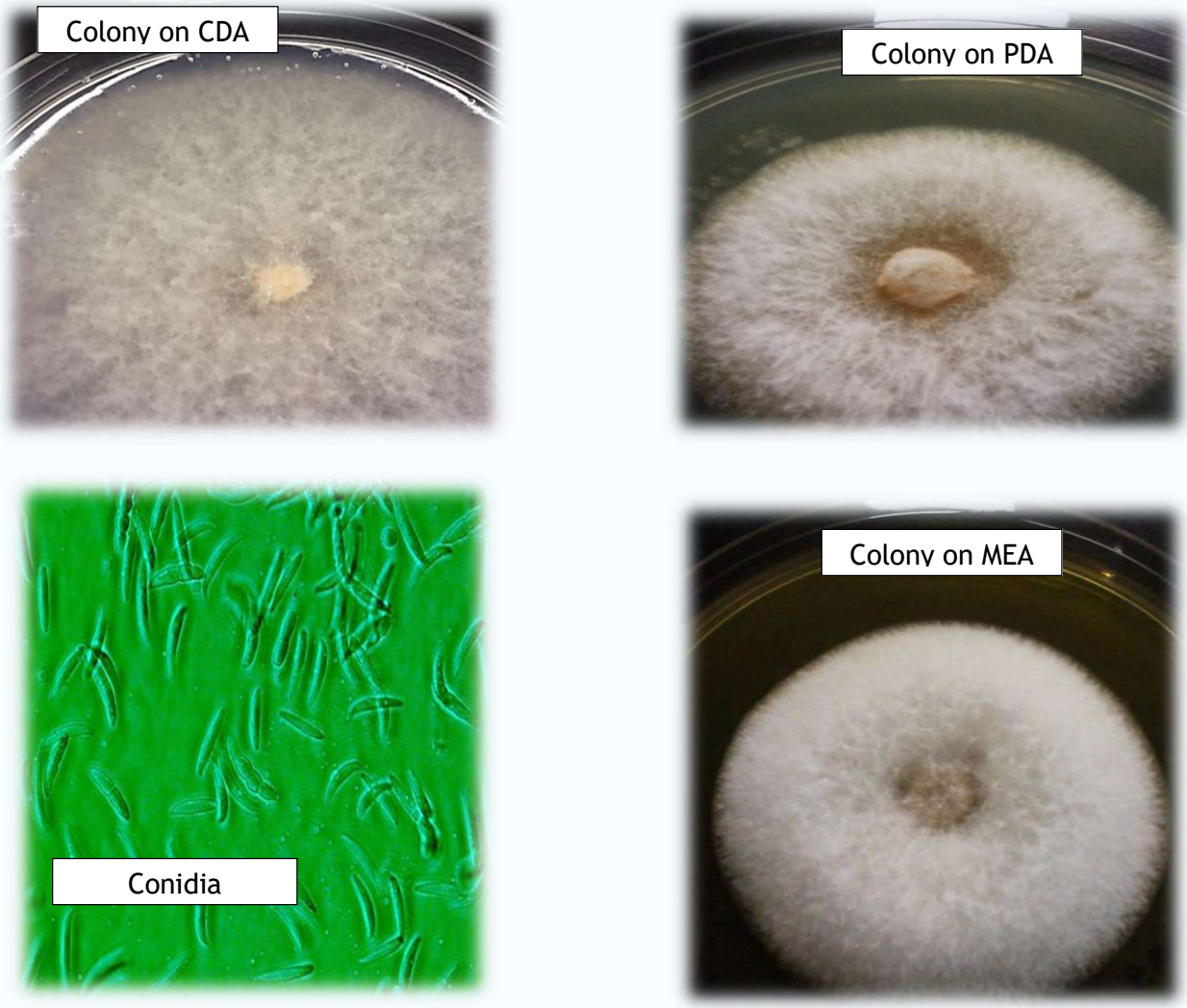

Figure (2): Morphology of *Fusarium* species at 27 °C for 5 days on three media.

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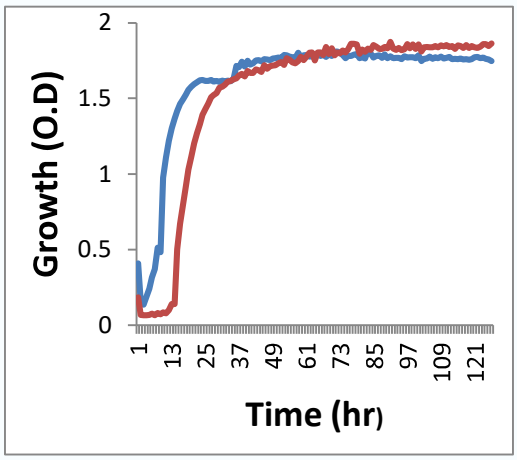

Figure (6): Growth of *Fusarium* species in PDB (blue) and PDB amended with magnesium chloride (red).

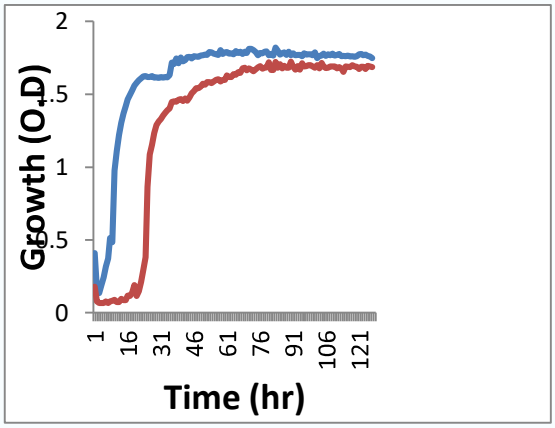

Figure (7): Growth of *Fusarium* species in PDB (blue) and PDB amended with KH_2PO_4 (red).

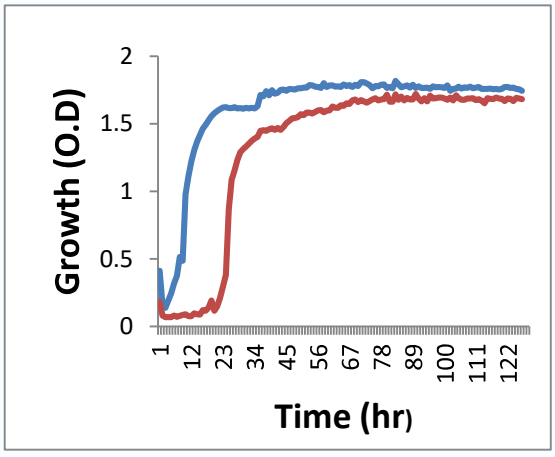

Figure (8): Growth of *Fusarium* species in PDB (blue) and PDB amended with Na_2HPO_4 (red).

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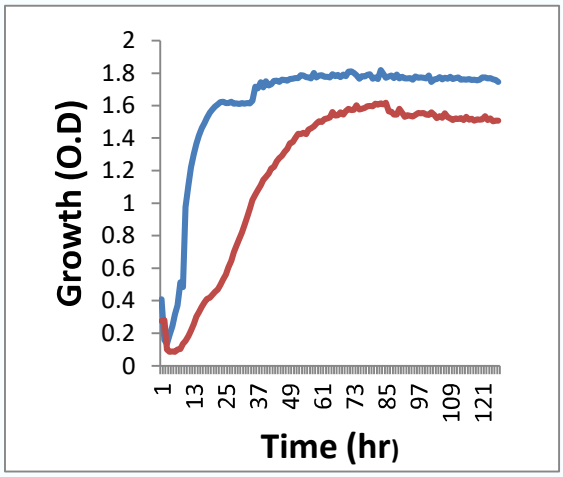

Figure (3): Growth of *Fusarium* species in PDB (blue) and PDB amended with aspartic acid (red)

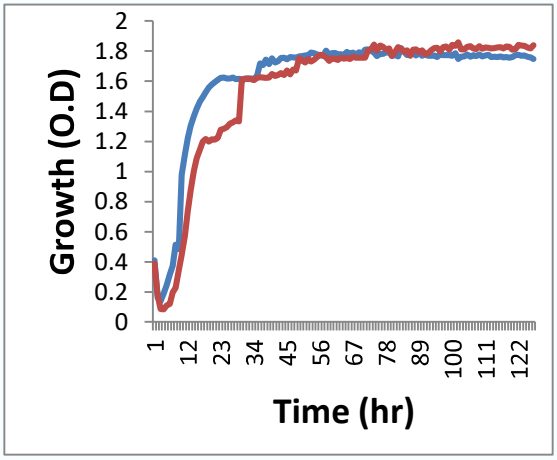

Figure (4): Growth of *Fusarium* species in PDB (blue) and PDB amended with potassium chloride (red).

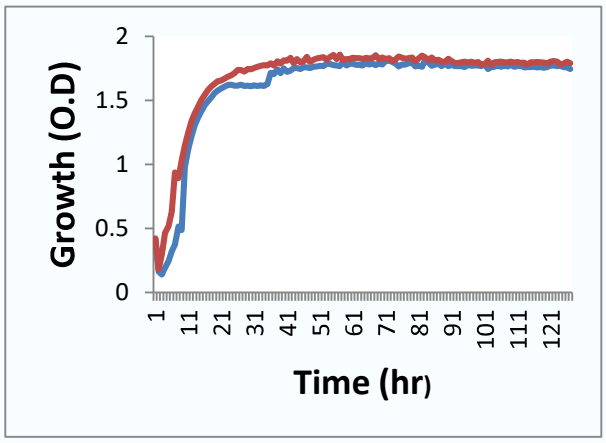

Figure (5): Growth of *Fusarium* species in PDB (blue) and PDB amended with magnesium sulfate (red).

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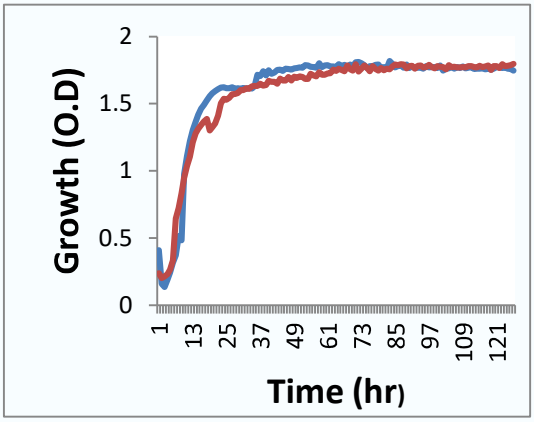

Figure (9): Growth of *Fusarium* species in PDB (blue) and PDB amended with thiamine-HCL (red).

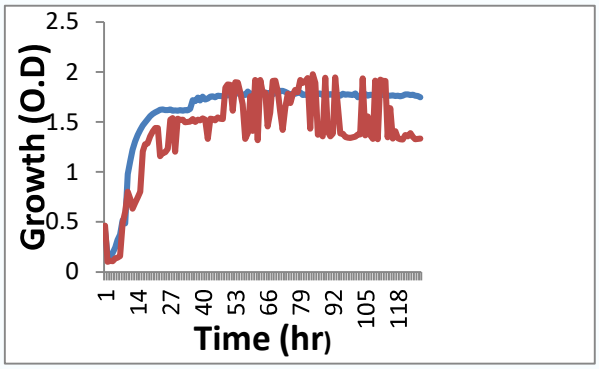

Figure (10): Growth of *Fusarium* species in PDB (blue) and PDB amended with ammonium chloride (red).

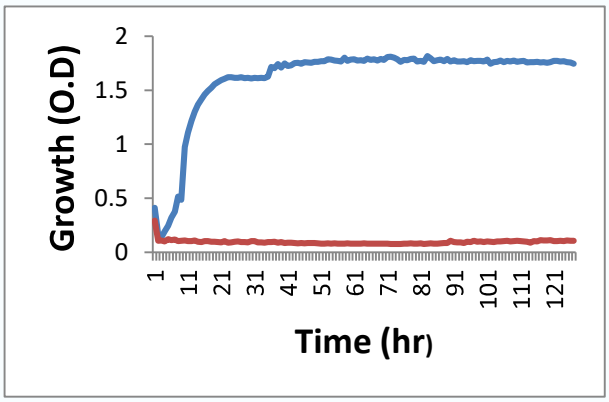

Figure (11): Growth of *Fusarium* species in PDB (blue) and PDB amended with ammonium acetate (red).

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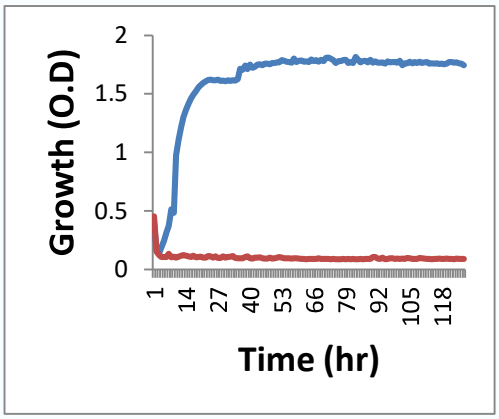

Figure (12): Growth of *Fusarium* species in PDB (blue) and PDB amended with potassium acetate (red).

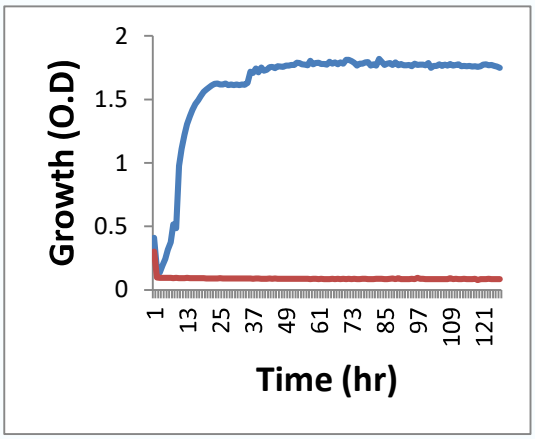

Figure (13): Growth of *Fusarium* species in PDB (blue) and PDB amended with zinc chloride (red).

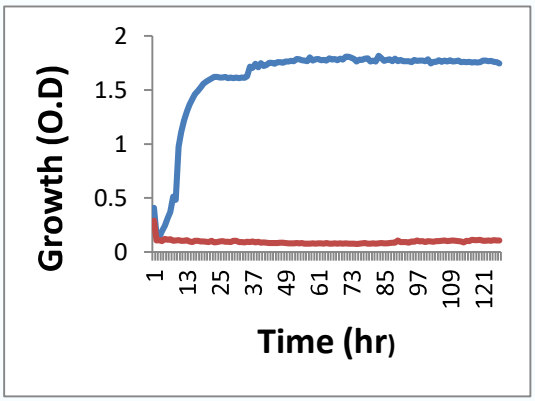

Figure (14): Growth of *Fusarium* species in PDB (blue) and PDB amended with zinc sulfate (red).