Effect Of Silicic Acid On The Growth Of *Fusarium* Species

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Abstract. This study was conducted to determine the influence of silicic acid on growth of *Fusarium solani, Fusarium oxysporum, Fusarium coeruleum* and *Fusarium ciliatum*. The addition of silicic acid to Czapek Dox liquid medium led to an increase in growth (biomass) of all *Fusarium* species over 4 weeks incubation period. The biomass increase was associated with increase in the concentration of soluble silicon, which also increased with increasing period of incubation. The largest amount of biomass was recorded in the treatments of *F. solani* and *F. oxysporum* that were five-fold higher than control. These species exhibited the largest concentration of soluble silicon present in the medium. Silicic acid stimulated the growth of *Fusarium* species when added to Czapek Dox liquid medium.

Key Words: Biomass, *Fusarium* species, Incubation, Silicon, Silicic acid.

Introduction

Silicon compounds, which are efficient at adsorbing gases and volatiles, remove combined carbon and nitrogen from the atmosphere, which then acte as nutrient source for bacterial growth. The possibility that bacteria can use silicon compounds as an energy source was suggested by Das *et al.*, (1992). Silicon is the second most common element on Earth after oxygen and is abundant in soils. Despite the fact that silicon is generally thought to be biologicallly inert, it is possible that soil microorganisms will have evolved some means of metabolising this element.

A few early studies began to reveal that a number of fungi appeared to be able to grow oligotrophically on nutrient-free silica gel. Except for studies on silicon accumulation by diatoms, the microbial metabolism of silicon has been largely ignored. The ability of fungi to grow on nutrient-free silica gel is now well-established (Alison, 1968; Morocha and Devay 1971; Ishida and Kadota 1981, Parkinson *et al.*, 1989; Barakah, 1992; Wainwright 1988; Wainwright 1993). Fungi and bacteria can solubilize insoluble silicates, a process which may be important in the biological weathering of rocks (Fry, 1990; Wainwright *et al.*, 1997). A wide range of fungi have been shown to be capable to grow oligotrophically, such as species of *Aspergillus, Trichoderma, Verticillium, Fusarium* and *Penicillium* (Tribe and Mabadeje 1972; Wainwright 1987; Parkinson *et al.*, 1989; Barakah 1992).

Oligotrophic growth of fungi has a number of important biotechnologically,

Fig. 1a: *F. solani*

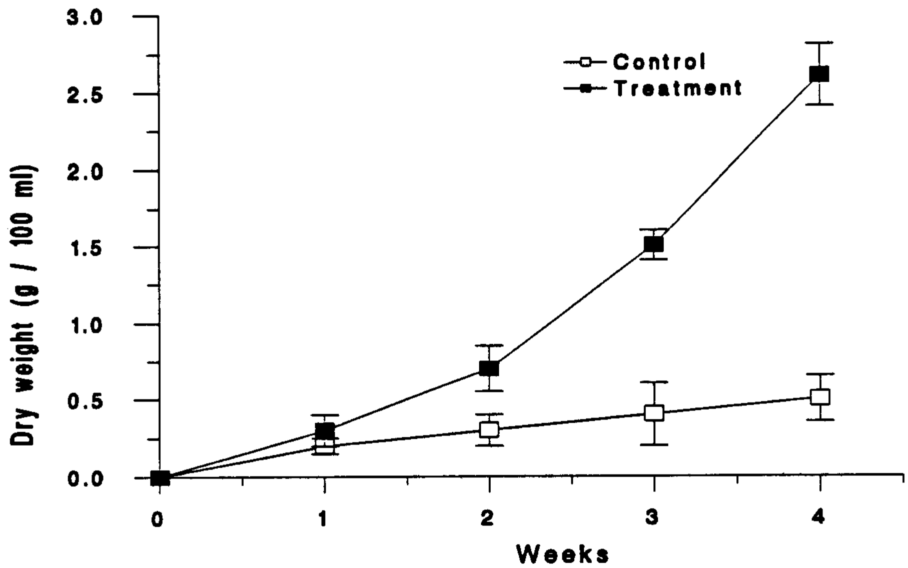

Fig. 1b: *F. solani*

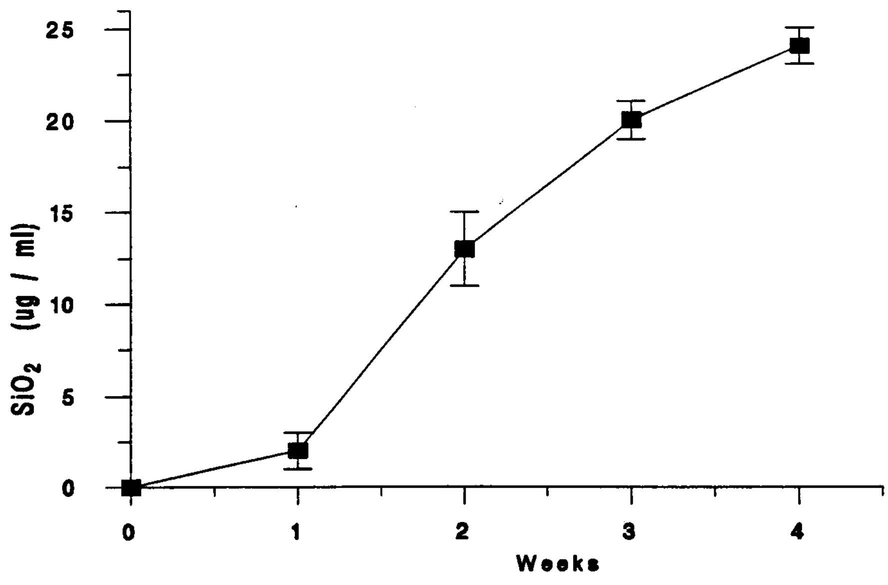

Fig 1a. Effect of silicic acid on growth of *Fusarium solani* in Czapek Dox liquid medium, all values are means of triplicats ± S.D. **Fig 1b.** Release of soluble silicon following growth of *Fusarium solani* in Czapek Dox liquid medium amended with silicic acid, all values are mans of triplicates ± S.D.

Fig. 2a: *F. oxysporum*

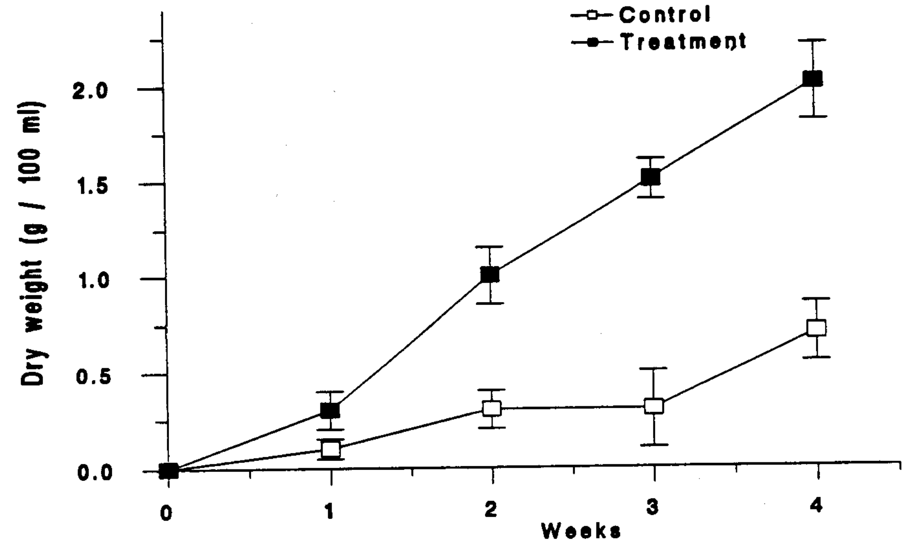

Fig. 2b: *F. oxysporum*

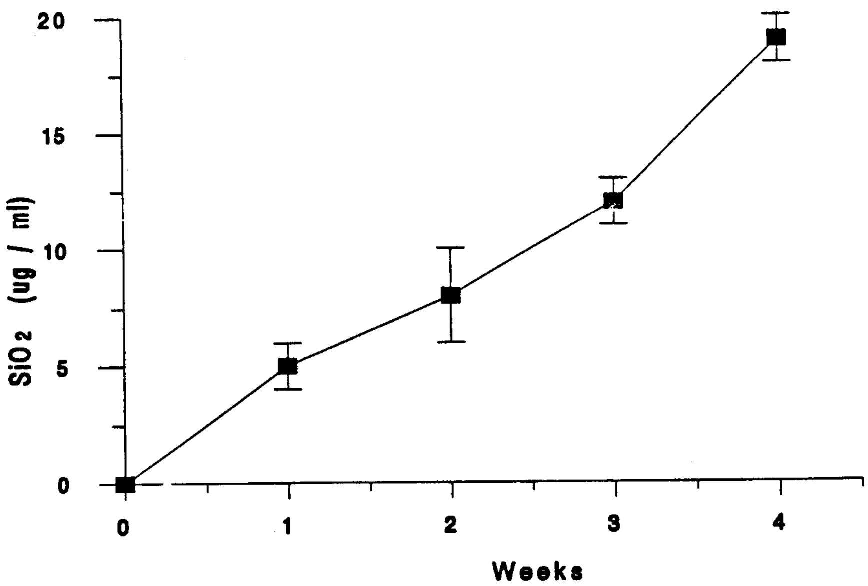

Fig 2a. Effect of silicic acid on growth of *Fusarium oxysporum* in Czapek Dox liquid medium, all values are means of triplicats ± S.D. **Fig 2b.** Release of soluble silicon following growth of *Fusarium oxysporum* in Czapek Dox liquid medium amended with silicic acid, all values are mans of triplicates ± S.D.

medical and environmental implications (Wainwright *et al.,* 1991). It is generally believed that fungi grow oligotrophically by using nutrients adsorbed by the silica gel from the atmosphere (Parkinson *et al.,* 1990; Parkinson *et al.,* 1991). It is possible, however, that silica gel itself increases hyphal growth or stimulates fungal spore germination.

The aim of the work reported here was to determine the effect of silicic acid on *Fusarium* species when growing in nutrient-rich Czapek Dox media. *Fusarium* species have been tested because of their high ability to grow oligotrophically (Johasson *et al.,* 1991, Barakah 1992).

Materials and Methods

Culture and growth medium

In this experiment *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* Schlecht., *Fusarium coeruleum* (Lib.) Sacc. and *Fusarium ciliatum* Link were obtained from the Sheffield University Animal and Plant Sciences Culture Collection. The *Fusarium* species were grown on Czapek Dox agar (Oxoid) fro 7 days at 25 °C. Aliquots of a suspension (1 ml) containing one of the *Fusarium* species were then used to inoculate Czapek Dox liquid medium (100 ml in 250 ml Erlenmeyer flasks), adjusted to pH 6.8 with 2N NaOH. The medium was then amended with 1.5 g of silicic acid (Sigma). All treatments were incubated for 4 weeks in triplicate on an orbital shaker (100 r.p.m.) at 25 °C. Controls lacking silicic acid, and containing silicic acid but not fungus, were also included.

After 7 days intervals the contents of the flasks were then filtered through pre-dried Whatman No. 1 filter paper and the dry weight of the mycelium retained by the filter paper was determined after drying to constant weight at 46 °C. The soluble silicon content of the medium was determined colorimetrically by adding the following to 1 ml of the filtrate: ammonium molybdate (2 ml, 10% v/v); ascorbic acid (2 ml, 5% w/v); oxalic acid (1ml, 10% w/v); and HCl (5 ml, 1:1 dilution of conc. HCl). After 15 min at room temperature, without shaking, the absorption of the blue colour was measured using spectrophotometer at 600 nm. The concentration of soluble silicon (as SiO_2) in the filtrate was then determined by reference to a standard curve prepared using EIL (BDH Chemicals, Poole, Dorset) standard silicon solution (Wainwright *et al.,* 1997).

Results

The addition of silicic acid to Czapek Dox liquid medium led to an increase in growth (biomass) of all *Fusarium* species over 4 weeks incubation period (Figs 1a, 2a, 3a and 4a); with biomass production increasing with increasing period of incubation. The biomass increase was associated with increase in the concentration of soluble silicon, which also increased with increasing period of incubation (Figs 1b, 2b, 3b and 4b). As can be seem, the significant increase of fungal biomass was started from the second week of incubation. Silicic acid clearly have a stimulatory effect on fungal growth.

Fusarium solani exhibited the highest growth (biomass) in Czapek Dox liquid medium amended with silicic acid which was 2.7 g/100 ml of media at the end of the incubation period followed by *Fusarium coeruleum* 2.6 g/100 ml of media. On the other hand the fungus of *Fusarium ciliatum*

Fig. 3a: *F. coeruleum*

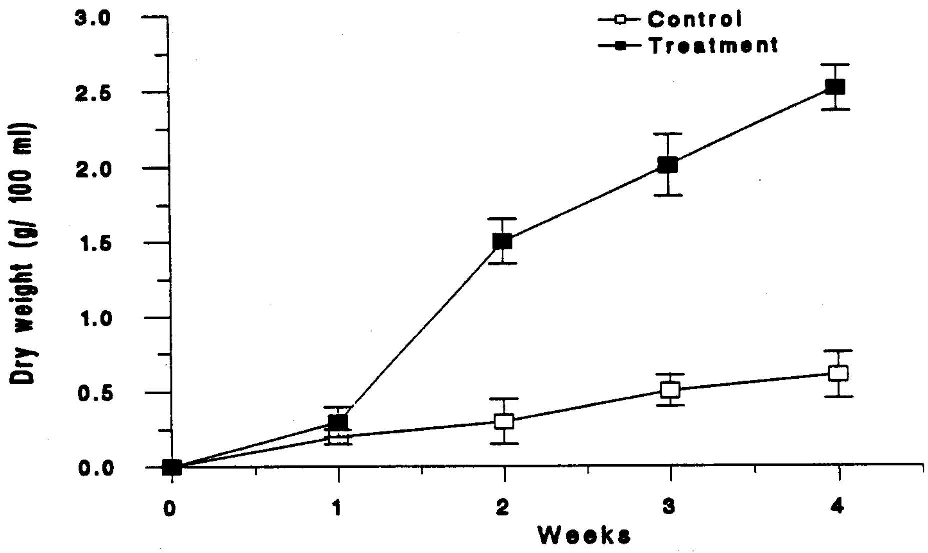

Fig. 3b: *F. coeruleum*

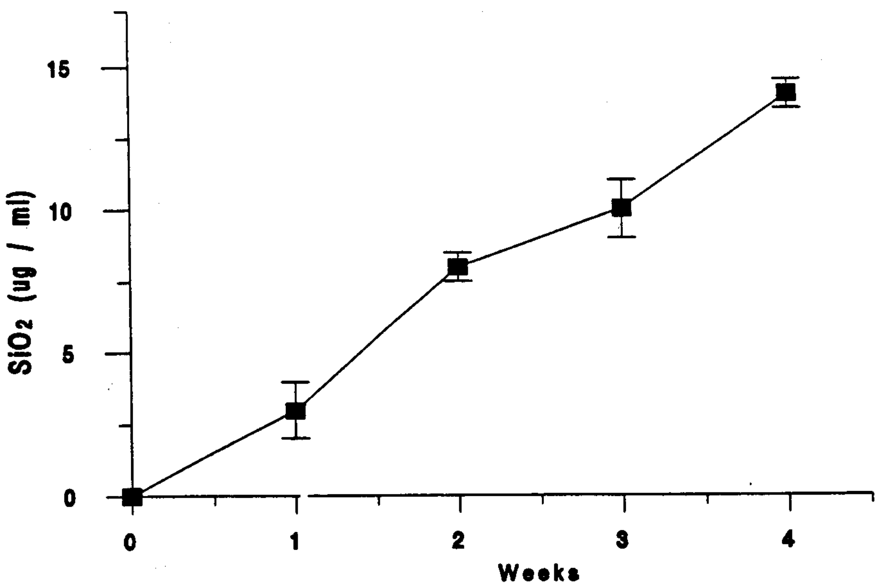

Fig 3a. Effect of silicic acid on growth of *Fusarium coeruleum* in Czapek Dox liquid medium, all values are means of triplicats ± S.D. **Fig 3b.** Release of soluble silicon following growth of *Fusarium coeruleum* in Czapek Dox liquid medium amended with silicic acid, all values are mans of triplicates ± S.D.

Fig. 4a: *F. ciliatum*

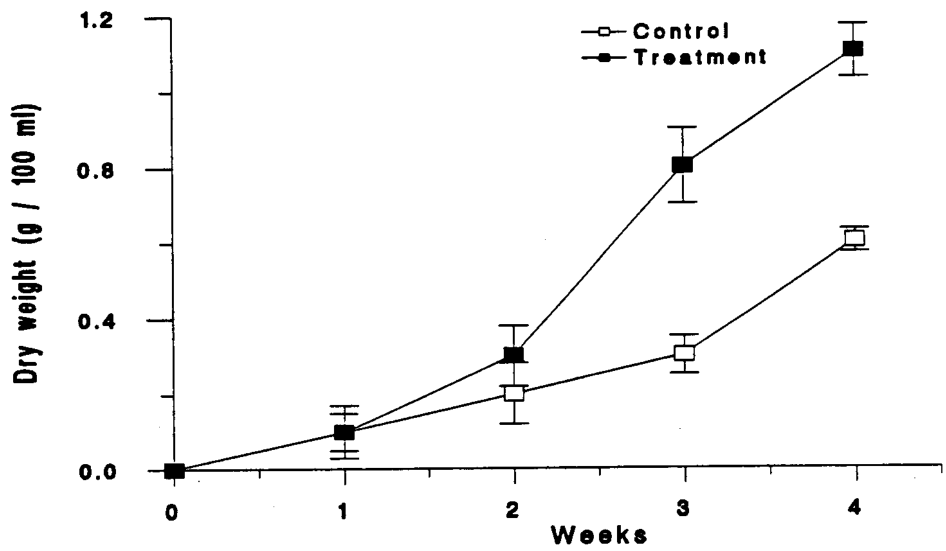

Fig. 4b: *F. ciliatum*

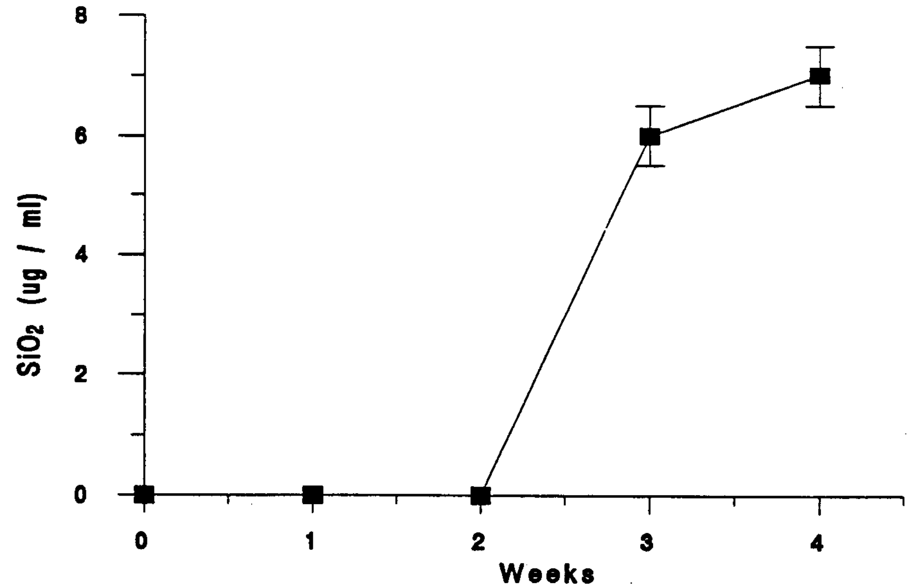

Fig 4a. Effect of silicic acid on growth of *Fusarium ciliatum* in Czapek Dox liquid medium, all values are means of triplicats ± S.D. **Fig 4b.** Release of soluble silicon following growth of *Fusarium ciliatum* in Czapek Dox liquid medium amended with silicic acid, all values are mans of triplicates ± S.D.

reached the lowest amount of biomass in the presence of silicic acid with 1.2 g/100 ml of media at the end of the incubation period.

All of *Fusarium* species tested solubized silicic acid leading to the release of soluble silicon (SiO_2) in Czapek Dox liquid medium (Figs. 1b, 2b, 3b and 4b). The release of soluble silicon was associated with a biomass increased of *Fusarium* sepcies. The species of *F. solani* followed by *F. oxysporum* have released the highest concentration of soluble silicon that were 25 and 19 μg/ml of SiO_2 respectively at the end of the incubation period (Figs 1b and 2b). While the lowest concentration of soluble silicon was observed in *F. ciliatum* with 8 μg/ml of SiO_2 at the end of the incubation period (Fig 4b). In addition the fungus *F. ciliatum,* failed to form soluble silicon until day 14, after which time the concentration of the SiO_2 increased as time of incubation increased. Therefore, *F. solani* and *F. oxysporum* were particularly active in silicic acid solubilization process forming the maximum amount of SiO_2 at the end of the incubation period.

Discussion

The presence of silicon in clays and other soils minerals may help explain why soil microorganisms, especially fungi, can grow in such an apparently nutrient-poor environment by: (i) using nutrients adsorbed by silicon compounds from the soil atmosphere, or (ii) by using energy derived from silicon metabolism to fix CO_2 chemoautotrophically or (iii) silicon may combined with other element acting as a source of energy (Alison 1968; Mirocha and Devay 1971; Wainwright 1988; Wainwright, 1993).

The results show that while all of *Fusarium* species can solubilize silicic acid, maximum solunilization was observed in the case of *F. solani.* These results also re-emphasize that the amount of soluble silicon present in the medium is related to the amount of biomass produced. A similar increase in growth of *Aspergillus oryzae* and the release of soluble silicon occurred in medium supplemented with silicic acid (Ishida and Kadota 1981, Parkinson *et al,* 1989; Wainwright *et al.,* 1997).

Silicon is generally thought to be biologically inert, it is possible that soil microorganisms will have evolved some means of metabolising this element (Alison 1968). It has also been suggested that bacteria use silicon-based autotrophy as a source of energy to support CO_2 fixation (Das *et al.,* 1992). Whatever the mechanism involved, it is clear that silicic acid, promotes fungal growth. Overall, the soluble silicon results were correlated with the biomass production of *Fusarium* species.

The largest amount of biomass was recorded in the treatments of *F. solani* and *F. oxysporum* that were five-fold higher than control. These species exhibited the largest concentration of soluble silicon present in the medium. Silicic acid stimulated the growth of *Fusarium* species when added to Czapek Dox liquid medium.

Silicic acid stimulated the growth of all *Fusarium* species tested leading to the release of soluble silicon (SiO_2) in Czapek Dox liquid medium.

References

Alison, A.C., 1968. Silicon compounds in biological systems. Proceedings of the Royal Society. 171, 19-30.

Barakah, F.N. 1992. Observations on the Oligotrophic Growth of Fungi. Ph.D. Thesis, Sheffield University, UK.

Das, S.; Mandal, S.; Chakrabarty, A.N. and Dastidar, S.G. 1992. Metabolism of silicon as a probable pathogenicity factor for *Mycobacterium. Indian Journal of Medical Research.* 95: 59-65.

Fry, J.C., 1990. Oligotrophs. In: Edwards, C.A. (Ed). Microbiology of Extreme Environments. Open University Press. Milton Keynes. Pp. 93-116.

Ishida, Y. and Kadota, H., 1981. Growth patterns and substrate requirements of naturally occurring obligate oligotrophs. *Microb. Ecol.* 7, 123-130.

Johasson, I.; Parkinson, S.M., Wainwright, M. and Killham, K. 1991. Oxidation of thiosulphate by *Fusarium oxysporum* grown under oligotrophic conditions. *Mycol. Res.* 95: 1169-1174.

Mirocha, C.A. and Devay, J.E. 1971. Growth of fungi on an inorganic medium. *Canadian Journal of Microbiology,* 17: 1373-1378.

Parkinson, S.M., Wainwright, M. and Killham, K. 1989. Observations on oligotrophic growth of fungi on silica gel. *Mycol.* 93: 529-534.

Parkinson, S.M., Killham, K. and Wainwright, M. 1990. Assimilation of $^{14}CO_2$ by *Fusarium oxysporum* grown under oligotrophic conditions. *Mycol. Res.* 94: 959-964.

Parkinson, S.M. Jones, R., Meharg, A.A., Wainwright, M. and Killham, K. 1991. The quantity and fate of carbon assimilated by $^{14}CO_2$ by *Fusarium oxysporum* grown under oligotrophic and near oligotrophic conditions. *Mycol. Res.* 95: 1345-1349.

Wainwright, M. 1988. Metabolic diversity of fungi in relation to growth and mineral cycling in soil. A review, *Trans. Brit. Mycol. Soc.* 90: 159-170.

Wainwright, M. 1993. Oligotrophic growth of fungi. Stress or natural state? Pp. 172-184 In: Jennings, D.H. (Ed.) *Stress Tolerance of Fungi.* Academic Press, London, UK.

Wainwright, M. Barakah, F. Al-Turk, I. and Ali, T.A. 1991. Oligotrophic microorganisms in industry, medicine and the environment. *Science Progress, Edinburgh* 75: 313-322.

Wainwright, M. Al-Wajeeh, K. and Grayston, S.J. 1997. Effect of silicic acid and other silicon compounds on fungal growth in oligotrophic and nutrient-rich media. *Mycol. Res.* 101: 933-938.

تأثير حمض السيليسيك على نمو أنواع فطر الفيوزاريوم

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قسم العلوم (الأحياء) – كلية المعلمين بالريـــاض
ص٠ب ٤٣٤١ – الرياض ١١٤٩١ – المملكة العربية السعودية

أجريت هذه الدراسة لتقدير تأثير حمض السيليسيك على نمو أنواع من فطر الفيوزاريوم هي:

Fusarium solani, Fusarium oxysporum, Fusarium coeruleum and Fusarium ciliatum

إضافة حمض السيليسك إلى وسط دوكس الغذائي السائل تؤدي إلى زيادة كبيرة في نمو جميع الأنواع خلال فـــترة التحضين التي تمتد إلى أربعة أسابيع. ووجد في هذه الدراسة أن الزيادة في النمو مرتبطة بالزيادة في تركيز السيليكون الذائب الذي هو أيضاً يزداد مع زيادة فترة التحضين. وقد سجلت العينة الملقحة بفطرة الفيوزاريـوم ســـولاني *Fusarium solani* وفطرة الفـــيوزاريوم أو كسيسبوريوم *Fusarium oxysporum* أكبر كيمة نمو (خمسة أضعاف) مقارنة بالتجربـــة الضابطـــة (الكونترول)، ويحتوي هذين النوعين على أعلى تركيز من السيليكون الذائب.