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Boron Tolerance and Accumulation in *Aspergillus flavus* and *Penicillium citrinum* Isolated from Saudi Arabian Soil

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Abstract. *Aspergillus flavus* and *Penicillium citrinum* survived in a growth medium containing up to 350 μg/ml boron. Tolerance and accumulation of boron in the tested fungi were studied. Stimulation of growth at 150 μg/ml boron and reduction at 350 μg/ml were evident. The present investigation is important in dealing with boron pollution in Saudi Arabia.

Introduction

Toxic effects of heavy metals on the growth of soil fungi isolated from different places in the world have described very well [1-11]. The total boron content of Saudi Arabian soil is in the range of 20 to 200 μg/g. Most soil boron is unavailable to plants. The available (hot water soluble) fraction ranges from 0.4 to 5 ppm [12]. Inorganic boron in soils is mainly in the form of tour-maline except in arid climates where borates of alkaline earth metals predominate. Boron is also added to soil to make boronated fertilizers [13].

Boron is important in plants metabolically and is believed to play a significant role in the translocation of sugar because the borate-polyhydric complex is more mobile than free polar sugar molecules. Most studies have examined the effect of boron on the metabolism of sugar beets, and it has been shown that an adequate boron supply is necessary for sugar synthesis [14].

Soil pH is one of the most important factors affecting the availability of boron, the lowest rate of boron uptake occurs when the soil pH is approximately 7 [14]. In alkaline soil the availability of boron increases with an increase in soil pH. This affects the boron health hazard problem, particularly in irrigated saline alkaline soil where the absorption of boron is temperature dependent and increases during warm periods.

Boron was examined in this study because of its importance in the natural environment, and its possible involvement in pollution in Saudi Arabia. This study is the first in a series aimed at examining the effect of heavy metals on the growth of some fungi isolated from Saudi Arabian soil.

Material and Methods

Soil analysis

Soil samples were collected from the industrial Yanbu city of Yanbu [15], from an exposed soil surface, in each case the soil was scraped (1 - 10 cm depth) into a plastic bag using a stainless steep spoon. On arrival in the laboratory all samples were passed through a 2.0 mm sieve. The pH value, total soluble salts and organic matter content were estimated in the soil samples according to the methods adopted by Black *et al* [16]. The soil type was determined by the hydrometer method as described by Piper [17]. Boron total content of the tested soil samples was measured according to Hashem [18].

Fungal isolation

A dilution plate method was used for the isolation of fungi tested in the present investigation as described by Johnson *et al.* [19]. Modified Czapek's medium was employed as recommended by Abdel-Hafez [20]. Rose bengal (1/5000) was used as a bacteriostatic agent [21]. Six plates were used for each sample, they were incubated at 30 °C for one week and were examined daily. Fungal species were identified according to Raper and Fennell [22] and Ramirez [23].

Metal tolerance and accumulation

Discs (7 mm diameter) of mycelium were cut from the margin of actively growing colonies of *A. flavus* and *P. citrinum* transferred to 100 ml conical flasks (1 discs/flask) containing malt extract medium (malt extract 5.0g, distilled water 1000 ml). Boron was added in the medium as B_2O_3 to give final concentrations of 0, 150, 250 and 350 μg/ml B in 50 ml of solution. The pH of the solution was adjusted to 5.0 before they were sterilized by filtration through millipore filters (0.45 μm). All flasks were incubated at 25 °C in the dark under static conditions. Mycelium were harvested at 10, 20, and 30 days. At harvest, mycelia were transferred to sheets of preweighed filter paper, thoroughly washed with deionized water, oven-dried at 80 °C for 24h and weighed. The boron concentration of mycelia was determined with atomic absorption spectrophotometer after nitric acid digestion [6]. The pH's of the residual media were also measured.

Results and Discussion

The results obtained show clearly that the organic matter and total soluble salts contents of the examined soil were generally low (Table 1) and this is in agreement with the findings of Abdel-Hafez [20] and Hashem [24] in some Saudi Arabia soils. The pH value of the examined soil was alkaline. Total boron content of the examined soil was 4.11 μg/g.

Table 1. Characteristics of examined soil (n = 5, ± standard deviation)

Soil type	Organic matter %	Total soluble salts %	Boron (μg/g dry weight)	pH
Sandy	0.51	0.23	4.11 ± 0.91	7.21

The type of soil used in this study was sandy. According to Boratynskia *et al.* [25] and Wells and Whitton [26], this type of soil usually contains the lowest amount of boron. Abdel-Hafez [20] and Hashem [24] found that to be the case in some samples of Saudi Arabian soil.

The growth curves for the tested fungi are given in Figs (1) and (2). Compared to the control, there was stimulation of growth at 150 μg/ml boron for both tested fungi. This is probably because boron is an essential element for microorganisms. Mechanisms of absorption and transport of boron in plants are well known [12], but little information is available about the mechanisms of absorption, transport and accumulation in microorganisms. Boron was reported to stimulate nitrogen fixation by bacteria. Also mycorrhizal plants have a greater need for boron than do non-mycorrhzal plants [7, p. 28].

There was a significant reduction in [illegible] wt yield at 250 and 350 μg/ml boron. The results were expressed in term of a tolerance index [6]. *P. citrinum* was more sensitive to boron than *A. flavus* (Fig. 3).

The results of the analysis of boron concentration in the mycelia of the tested fungi are presented in Fig. (4). Mycelia of *A. flavus* accumulated boron to a maximum of 5200 μg/g dry weight of mycelium, in the presence of 350 μg/ml boron, while *P. citrinum* accumulated 4000 μg/g at 350 μg/ml. This was after 30 days growth for both tested fungi. It appeared that boron must have been transported in high amounts into mycelium because at a high external boron concentration, mycelial growth was inhibited. The pH in these tests declined steadily at all concentrations. The fall in pH was broadly in proportion to the original boron concentrations, the highest metal level giving the maximum reduction of pH (Table 2).

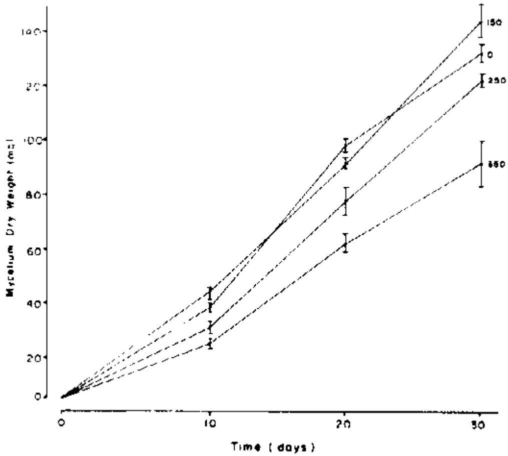

Fig.1. Mycelium dry weight (mg) of *A. flavus* from cultures with different concentrations of B (μg/ml). The values are the means of 5 replicates. Vertical bars represent 95% confidence limits.

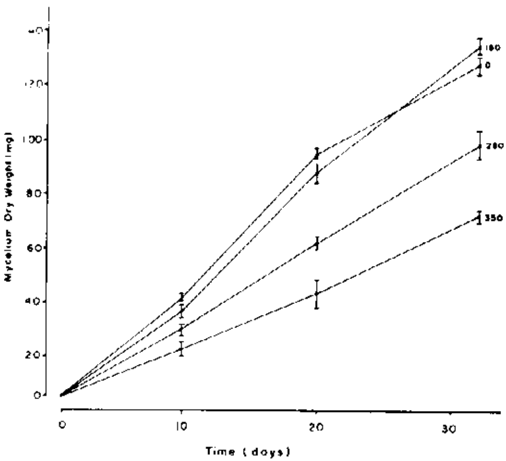

Fig.2. Mycelium dry weight (mg) of *P. citrinum* from cultures with different concentrations of B (μg/ml). The values are tne means of 5 replicates. Vertical bars represent 95% confidence limits.

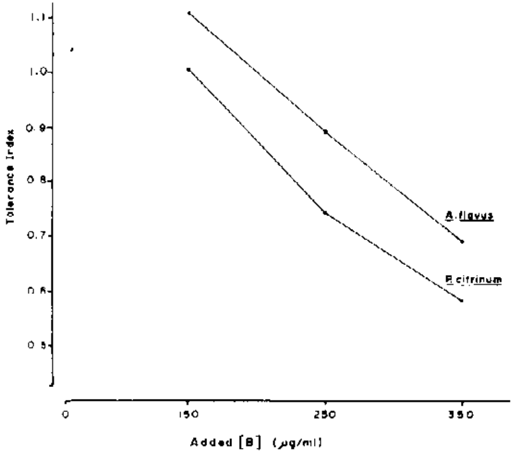

Fig.3. Tolerance index from mycelium dry weight (mg) and boron concentrations (μg/ml). After 30 days growth of the tested fungi.

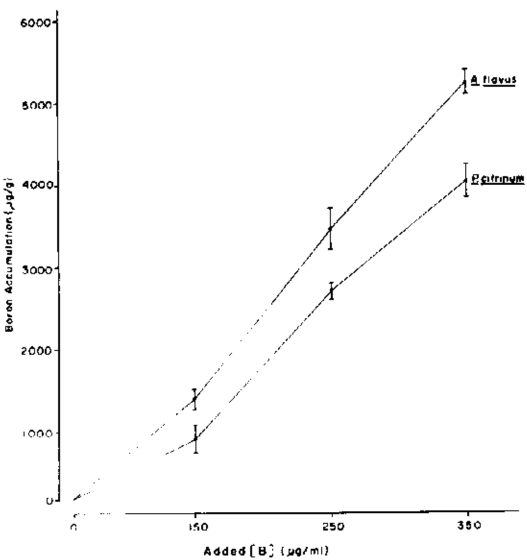

Fig.4. Boron concentrations in the mycelium of the tested fungi (μg/g dry weight) after 30 days. The values are the means of 5 replicates. Vertical bars represent 96% confidence limits.

Table 2. The final pH of the growth medium modified Czapek's after different periods of incubation of the tested fungi (n = 5, start ph = 5.0) in the presence of different amount of boron

Fungus	Time (days)	Added boron (μg/ml)			
		0	150	250	350
		pH			
A. flavus	10	4.81	4.70	4.52	4.61
	20	4.60	4.53	4.48	4.45
	30	3.48	3.39	3.40	3.10
P. citrinum	10	4.63	4.43	4.23	4.01
	20	4.51	4.20	4.01	4.13
	30	3.31	3.22	3.11	3.08

Previously there was no information concerning the tolerance for and accumulation of boron in fungi isolated from Saudi Arabia. According to Hashem [6], *A. niger* and *P. chrysogenum* isolated from Taif, Saudi Arabian soil were resistant to copper and accumulated more than 5500 μg Cu g dry biomass in the presence of 500 μg Cu ml^{-1}.

Soluble forms of boron are readily available to plants which can take up undissociated boric acid as well as other boron compounds present in the ambient solution. The property of boric acid to complex with polysaccharides is believed to play an important role in passive absorption. The physiological role of boron differs from other micronutrients in this anion has not been identifed as a component of any specific enzyme, and biochemical role of this element is still not well understood [27]. Much remain to be done on the genetic, physiological and metabolic basis of resistance of microorganisms to boron.

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مقاومة تراكم عنصر البورون بوساطة الفطرة اسبرجيلس فلافوس والفطرة بنيسليام ستربنوم المعزولتان من تربة المملكة العربية السعودية

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(استلم في ١٧/٩/١٤١٣هـ؛ قبل للنشر في ١٣/١١/١٤١٤هـ)

ملخص البحث. أثبتت نتائج هذه الدراسة أن الفطرة اسبرجيلس فلافوس والفطرة بنيسليام ستربنوم المعزولتان من تربة المملكة العربية السعودية ذات قدرة على النمو ومقاومة التراكيز المختلفة من عنصر البورون والتي تصل إلى ٣٥٠ جزء في المليون في البيئات السائلة. وقد تمت مناقشة ميكانيكية المقاومة والامتصاص لعنصر البورون على ضوء النتائج المتحصل عليها مقارنة بالأبحاث التي أجريت في هذا المجال في أماكن مختلفة من العالم.

وجد أن تركيز ١٥٠ جزء في المليون من عنصر البورون ذو أثر تحفيزي على نمو الفطريات المستخدمة في هذه الدراسة بينما تركيز ٣٥٠ جزء في المليون ذو أثر تثبيطي على النمو. وقد تمّ عرض النتائج المتحصّل عليها باستخدام طريقة الوزن الجاف ومنحنى المقاومة والامتصاص والتغير في الرقم الهيدروجيني.

هذه الدراسة تعتبر مهمة للحّد من التلوث بالبورون باستخدام بعض الفطريات القادرة عند نموها على امتصاص عنصر البورون في المملكة العربية السعودية.