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The influence of tin ions on growth and enzymatic activity of entomopathogenic fungi

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Abstract: In this *in vitro* study, the influence of tin ions at concentrations of 1-1000 ppm on the development and enzymatic activity of four entomopathogenic fungi (*Beauveria bassiana*, *Beauveria brongniartii*, *Isaria fumosorosea* and *Metarhizium robertsii*), that are commonly used in biological plant protection, are examined. Each of the fungal species tested reacted differently to contact with the Sn^{2+} ions at the tested concentrations. Exposure to Sn^{2+} ions affected the rate of development, morphology and enzymatic activity of fungi. Of the four fungal species studied, *M. robertsii* was the most resistant and showed complete growth inhibition at the highest Sn^{2+} concentration tested (1000 ppm).. For the other entomopathogenic fungi, the fungicidal effect of Sn^{2+} ions was noted at the concentration of 750 ppm. Exposure to Sn^{2+} ions (up to 500 ppm) resulted in enhanced biochemical activity; and all entomopathogens that were tested showed increased production of N-acetyl-β-glucosaminidase (NAG) as well as several proteases. Moreover, *B. brongniartii* and *M. roberstii* showed increased lipases synthesis. These changes may increase the pathogenicity of

the fungi, thereby making them more effective in limiting the population of pest insects. The exposure of the entomopathogenic fungi to a medium containing Sn^{2+} ions, at concentrations that were appropriate for each species, induced hyperproduction of hydrolases, which might be involved in aiding the survival of entomopathogenic fungi in the presence of heavy metals. This study shows that the fungistatic effect of Sn^{2+} on entomopathogenic fungi did not restrict their pathogenicity, as evidenced by the stimulation of the production of enzymes that are involved in the infection of insects.

Keywords: tin; heavy metals; entomopathogenic fungi; enzymatic activity

Running head: The influence of tin on entomopathogenic fungi

Introduction:

Metals are an integral part of all ecosystems and occur in both elemental and ore forms throughout nature. Industrialization and urbanization, in this and preceding centuries, have generated a tremendous amount of soil, water, and air pollution, which interferes with homeostasis in the ecosphere [1]. The utilization of pesticides, chemical fertilizers, and preservatives, particularly in agriculture, contributes to fluctuations in the chemical composition of the ecosphere and to the disturbance of interactions between organisms in the soil [2]. Life has evolved in environments that are rich in various metals and all cells have incorporated metal ions into their essential cellular functions [3]. Consequently, life forms that are continuously exposed to potentially toxic conditions have evolved mechanisms of metal homeostasis and metal resistance to adapt to the metals that are present in their environments. This requires mechanisms that ensure sensitivity towards different metal species at the concentration at which they are present in the environment [3]. The introduction of heavy metal compounds into the environment generally induces morphological and physiological changes in microbial communities [4]. It is well established that heavy metals

interfere with the physiological, enzymatic, and reproductive processes of organisms, thereby affecting the ecosystem.

Entomopathogenic fungi (EPF) are a group of highly specialized microorganisms that can infect arthropods, including insects that are pests of crop plants [5]. The ability of EPF to infect insect pests makes this group of fungi particularly important in biological plant protection [6]. EPF occur primarily in the soil and constitute an essential part of the organic biomass [7,8]. Soil can provide a substrate for the maintenance of a natural reservoir of many EPF. For this reason, soil can be inoculated with EPF by an infected insect entering the soil, by the deposition of spores on the surface of the soil or by natural dispersion mechanisms [9]. The presence and pathogenicity of many EPF depend on their interactions with host organisms, prevailing climatic conditions, as well as other biotic and abiotic factors, which include contact with heavy metals. Laboratory studies have shown that heavy metals influence growth, metabolism, and pathogenicity of EPF [2,6,10-12].

Tin (Sn) is a naturally occurring heavy metal that is present at an average concentration of 2 mg/kg in the Earth's crust. The concentration of Sn in the environment is, however, highly variable and is dependent both on the use of Sn and the release of Sn from Sn-containing entities. The release of Sn can occur by natural means such as the weathering of rocks or volcanic eruptions or due to anthropogenic activities, such as industrial processes, agriculture, and mining [13]. Normal concentrations of Sn in unpolluted soils range from <1 mg/kg to 200 mg/kg; the Sn present in the soil occurs in two oxidative states (II and IV). In the soil, Sn usually has limited mobility and is usually tightly bound in the top soil [14]. However, due to the increase in anthropogenic activities such as agriculture, which release Sn products into the environment, concentrations of Sn may be elevated in certain areas [14]. Sn can combine with chemicals like chlorine, sulfur, or oxygen to form inorganic Sn compounds (i.e. chlorides, sulfides and oxides) [15]. Inorganic Sn compounds are used as pigments in the

ceramic and textile industry. Tin (II) chloride, $SnCl_2$, is used as a protective tinplate coating for steel sheet for use in manufacturing, processing, and packaging of foods as well as in biocidal preparations. Sn is used in canned foods to protect the steel base from corrosion both externally, due to aerobic conditions, and internally, when the Sn is under anaerobic conditions and in contact with food. The Sn in canned food is likely to be in the inorganic salt form as opposed to the covalently-bound organometallic forms. The corrosion of cans may be one of ways in which Sn is released into the environment as pollution. Although the biological functions of Sn have not been described to date, it is difficult to agree with the opinion that Sn is a non-essential metal that is of no importance to organisms [15,16]. Sn compounds affect the physiology of bacteria and fungi [17-21], plants [13,14], and animals [15,17,22,23]. Interactions of Sn with microorganisms are ambiguous, as although Sn and its compounds can be metabolized by some microorganisms, they are toxic to others. Microbial interactions with Sn are important, because microbes are at the base of many food webs and are likely to be significant in the environmental transformation of Sn compounds. This suggests that microbes may have significant potential in the remediation of Sn-polluted waste streams and of Sn-polluted ecosystems [24].

Little is known at this time about the effect of Sn on the growth and biochemical activity of EPF. This study aims to determine the sensitivity of four EPF (*Beauveria bassiana*, *Beauveria brongniartii*, *Isaria fumosorosea*, and *Metarhizium robertsii*) to increasing concentrations of Sn^{2+}. In addition, this study aims to understand the response of the EPF to Sn, which determines their potential of EPF in biological pest control.

Materials and methods:

Fungal strains

Beauveria bassiana (Bals.-Criv.) Vuill. (UPH34), *Beauveria brongniartii* (Sacc.) Petch (UPH42), *Isaria fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (UPH62) and *Metarhizium robertsii* J.F. Bisch., S.A. Rehner & Humber (WA27856) fungal strains were obtained from the Fungal Collection at the Department of Plant Protection and Breeding, Siedlce University of Natural Sciences and Humanities (Siedlce, Poland). The strains were isolated near Siedlce (Masovian district, Poland) from the soil of arable fields by using the *Galleria* bait method [25]. Before the experiments, all isolates were grown on a Sabouraud medium (Biocorp, Poland) and stored at 4°C.

Media and the preparation of EPF inocula

The influence of metal on EPF was tested on solid PDA medium (Biocorp, Poland) supplemented with Sn (II) chloride (Sigma-Aldrich). $SnCl_2 \cdot 2H_2O$ salt was added to PDA medium after autoclaving (when the temperature of the medium reached approx. 50ºC) to achieve concentrations of 1, 10, 50, 100, 250, 500, 750 and 1000 ppm of Sn^{2+} ions. The medium was then placed on a magnetic stirrer and when it cooled, it was poured into 90 mm Petri dishes. The PDA medium, which lacked added Sn^{2+} ions served as the control medium. Inocula were prepared from 10-day old fungal colonies grown on pure PDA medium. Aerial hyphae of EPF strains were collected and a suspension of fungal spores in sterile physiological saline was prepared. The concentration of fungal spores was calculated using a Thoma counting chamber and was approx. 1,0 x 10^9/mL. A twenty µL drops of EPF suspensions were transferred using an automatic pipette to the center of the test plates. Five biological repeats were prepared for each Sn concentration as well as for the control, and the plates were incubated at 25°C for 18 days.

Growth response studies and the determination of the minimum inhibitory concentration of Sn

The development of fungi was evaluated using the tolerance index (TI) as previously described [26]. To compare the TI of the EPF strains, the radius of the colony extension on PDA medium supplemented with Sn^{2+} ions at different concentrations was measured against the control medium (PDA devoid of added Sn^{2+} ions). The radial growth was evaluated by performing four measurements in millimeters, each measurement passed through the center of inoculated EPF material. The minimum inhibitory concentration (MIC) was defined as the minimum inhibitory concentration of heavy metal in the medium that inhibited the visible growth of tested EPF strains. If no fungal growth was observed after the incubation period, that Sn^{2+} ion concentration was considered the highest metal concentration tolerated by the EPF tested.

The determination of fungal enzymatic activity

The API-ZYM test (bioMérieux, Lyon, France) was used to semi-quantitatively determine the activity of 19 hydrolytic fungal enzymes including alkaline phosphatase (2), esterase (C4) (3), esterase lipase (C8) (4), lipase (C14) (5), leucine arylamidase (6), valine arylamidase (7), cystine arylamidase (8), trypsin (9), chymotrypsin (10), acid phosphatase (11), naphthol-AS-BI-phosphohydrolase (12), α-galactosidase (13), β-galactosidase (14), β-glucuronidase (15), α-glucosidase (16), β-glucosidase (17), *N*-acetyl-β-glucosoaminidase (NAG, 18), α-mannosidase (19) and α-fucosidase (20) according to the manufacturer's instructions. Fungal cultures that were grown on PDA without and with Sn^{2+} ions at concentrations of 1, 100 and 500 ppm for 14 days were transferred into a sterile physiological saline solution. The API-ZYM strips were inoculated with the resuspended EPF culture and then incubated at 37°C for 4 h. Hydrolytic activity was determined in nanomoles (nM) of hydrolyzed substrate in a 5-grade color scale, ranging from 0 to 5, as described by

manufacturer. A 0 indicates a negative reaction with no enzyme production, 1 indicates 5 nM hydrolyzed substrate, 2 indicates 10 nM hydrolyzed substrate, 3 indicates 20 nM hydrolyzed substrate, 4 indicates 30 nM hydrolyzed substrate and 5 indicates 40 nM or more of hydrolyzed substrate.

Results:

Compared to the control, all EPF species tested showed a delay in linear growth on PDA medium supplemented with Sn^{2+} ions at concentrations of 1-500 ppm (Figs.1, 2, 3 and 4). The complete inhibition of *B. bassiana*, *B. brongniartii* and *I. fumosorosea* development occurred at an MIC of 750 ppm whereas inhibition of *M. robertsii* development occurred at an MIC 1000 ppm.

Exposure to Sn also resulted in morphological changes in the EPF mycelia. At a concentration of 250 and 500 ppm, there was a reduction in the aerial hyphae of *M. roberstii*. At Sn^{2+} concentrations higher than 500 ppm, the aerial hyphae were no longer visible for *B. bassiana* and *B. brongniartii*. There were no morphological changes in the mycelia of *I. fumosorosea* regardless of the Sn^{2+} concentration present in the growth medium, the hyphae were indistinguishable from those that developed under control conditions. The exposure of *B. bassiana* colonies to PDA medium containing Sn^{2+} concentrations between 1-50 ppm resulted in the formation of a white halo around the fungal colonies. In contrast, a pink pigment and a dark halo was produced around *B. brongniartii* colonies on PDA plates supplemented with 1 and 10 ppm of Sn^{2+}. No color reactions were observed around *I. fumosorosea* and *M. robertsii* colonies.

The changes in the concentration of Sn^{2+} ions resulted in changes in the enzymatic activity of the EPF tested (Tab. 1). When compared to the enzymatic activity on control media, the growth of *B. bassiana* in the presence of Sn^{2+} at a concentration of 1 ppm did not result in the

inhibition of synthesis of any enzyme. In contrast, increased production of leucine arylamidase (6), valine arylamidase (7), acid phosphatase (11), α-galactosidase (13) and NAG (18) was detected. At a concentration of 500 ppm Sn^{2+}, increased activity of acid phosphatase (11), naphthol-AS-BI phosphohydrolase (12) and β-glucosidase (17) was detected when compared to the control *B. bassiana* sample. However, compared to the activity detected at 1 ppm Sn^{2+}, decreased leucine arylamidase (6) and NAG activities (18) were detected.

The growth of *B. brongniartii* in medium containing 1 ppm of Sn^{2+} ions resulted in increased alkaline phosphatase (2), lipase esterase (C8) (4), acid phosphatase (11), α-galactosidase (13), β-galactosidase (14), α-glucosidase (16), β-glucosidase (17), NAG (18) and α-mannosidase (19) activity but reduced esterase (C4) (3) and cystine arylamidase (8) activity compared to those of the control sample. Growth in medium containing 100 and 500 ppm Sn^{2+} ions resulted in increased synthesis of leucine arylamidase (6), valine arylamidase (7), acid phosphatase (11), naphthol-AS-BI-phosphohydrolase (12), β-galactosidase (14), β-glucosidase (17) and NAG (18).

Growth of *I. fumosorosea* in medium containing a concentration of 1 ppm Sn^{2+} resulted in a reduction in naphthol-AS-BI-phosphohydrolase (12), β-galactosidase (14) and β-glucosidase levels compared to those in the control. The other enzymes were produced at the same levels in the control and Sn^{2+} containing medium. The development of *I. fumosorosea* in medium containing Sn^{2+} at 100 and 500 ppm resulted in increased levels of cystine arylamidase (8), naphthol-AS-BI phosphohydrolase (12), NAG (18), and α-fucosidase (20) compared to those in the control.

When compared to the control sample, the growth of *M. roberstii* on media containing different concentrations of Sn^{2+} ions resulted in increased activity of the majority of the tested enzymes. When exposed to media containing 1 and 100 ppm Sn^{2+} ions, no enzyme was

inhibited; however, the levels of acid phosphatase (11) and naphthol-AS-BI phosphohydrolase (12) and NAG (18) were elevated compared to those in the control. The synthesis of these enzymes was limited when the medium contained Sn^{2+} ions at a concentration of 500 ppm; however, the levels remained higher than those found in the control.

Discussion

There are few studies that have focused on the toxicity of inorganic Sn compounds towards microorganisms. This may be due to the widespread belief that inorganic Sn species hydrolyze to form insoluble and nontoxic Sn oxides or Sn hydroxides. Most of the studies that have studied the toxicity of Sn have concentrated on organotin compounds [27]. The mechanisms by which inorganic Sn exerts its toxic effects, and the influence of these compounds on fungal physiology, is unclear. It is possible that it may be complex to understand. Tobin and Cooney [17] observed that the inorganic Sn ions (Sn^{2+} and Sn^{4+}) bind to *Candida maltosa* yeast cells at levels of 0.3 and 0.23 mM Sn/g cells, respectively however Sn did not inhibit growth of the *C. maltosa* at concentrations up to 0.8 mM. The inorganic Sn did not cause the leakage of potassium from the yeast cells however organotins did affect the physiological state of yeast. With reference to Basidiomycota, Kähkönen *et al.* [28] showed that although there are individual species of fungi with a high tolerance towards inorganic Sn compounds ($SnCl_4 \cdot 5H_2O$), there are many more species that lack this tolerance.

It has been reported that $SnCl_2$ is capable of promoting the formation of reactive oxygen species (ROS), which are responsible for the oxidative stress that causes DNA damage [29]. According to Dantas *et al.* [30], the Sn^{2+} toxicity mechanism may be related to Fenton-like reactions. ROS, such as the hydroxyl radical ($\cdot OH$), which is produced in the cells, is capable of damaging important cellular targets, including membranes and DNA. Metal ions, including a number of transition metals such as iron, copper, zinc, and chromium,

are able to mediate Fenton or Fenton-like reactions that generate ROS in the presence of hydrogen peroxide (H_2O_2) [30]. At higher concentrations, some of the metal ions, and particularly the heavy metals, interfere negatively with cellular metabolism as they may inactivate proteins and damage DNA [31]. The genotoxic potential of Sn may also be significantly modulated by other non-DNA repair or membrane transport-related physiological parameters. This includes the quality and quantity of enzymatic and non-enzymatic scavengers of metal-induced ROS, which may be a crucial factor that influences the physiological response of EPF. Inorganic Sn increases the frequency of chromosomal aberrations, sister chromatid exchange and reduces cell proliferation [15,32]. Inorganic Sn also induces rapid and prolonged suppression of DNA synthesis resulting in changes in gene expression as reported by McLean *et al.* [33]; these authors also noticed that $SnCl_2$ produced single-strand breaks in DNA. The toxicity of inorganic Sn ($SnCl_2$) has also been demonstrated towards microorganisms that live in saline estuaries. Hallas *et al.* [27] suggest that this may be due to the interaction of the metal with polysaccharides and the consequent formation of cytotoxic complexes.

This study also demonstrated the toxicity of Sn^{2+} ions to microorganisms. This finding is confirmed by the individual reactions of EPF to Sn that were observed in this study. EPF exhibited differential tolerance to the presence of Sn, there were visible differences in development as well as differences in EPF biochemical activity; these demonstrate that Sn exerted a degree of toxicity towards microorganisms. The morphological changes and the colorful reactions that occur when EPF are in contact with Sn^{2+} ions may be the result of physiological disturbances; this has been shown in other microorganisms [26,34,35]. The production of organic acid-based pigments (such as salts of oxalic acid and citric acid), in fungal cells or their secretion into the environment could be harnessed to precipitate metal ions; this could be used in mechanical detoxification.

There are two mechanisms that have been proposed to explain the tolerance of fungi to heavy metals. These include the extracellular sequestration of the metal ions by chelation or cell wall binding and the intracellular and physical sequestration of metals by binding them to proteins or other ligands to prevent the metal ion from damaging the metal sensitive cellular targets [34,35]. Among the tested EPF, the highest tolerance to contact with Sn^{2+} was demonstrated by *M. robertsii*, with an MIC value of 1000 ppm. For the other EPF species, the MIC values were 750 ppm. The fungistatic effect of the Sn^{2+} ions is thought to be due to the inhibition of the logarithmic phase of fungal growth, specifically the trophophase, which is usually dependent on environmental conditions [1,26,34,36]. The consequence of the Sn-dependent physiological changes is the delay in the stationary phase, the idiophase, which is important for fungi because it is associated with the production of key secondary metabolites, such as mycotoxins [37]. Limiting the toxigenic potential of EPF may reduce their pathogenicity to insects.

Insect mycoses are, however, also dependent on other factors that interact with or are independent of mycotoxins. In addition to their mycotoxin-forming ability, the effectiveness of entomopathogens in the reduction of insect populations is also determined by the activity of their enzymes, especially those that are involved in the degradation of the epicuticle, which is the outer body of the insect. These enzymes include those that are active in the digestion of chitin, which constitutes 60% of the dry matter of insects' epicuticle. Chitin is composed of polymers of *N*-acetyl-D-glucosamine whose structure is destroyed by the enzyme NAG. The present study indicates an interesting and as yet unreported effect of Sn; stimulation with Sn resulted in the elevated production of this enzyme by *B. bassiana* (at 1 ppm), *B. brongniartii* (at 1, 100 and 500 ppm), and *M. robertsii* (at 1 and 100 ppm). Overproduction of NAG was also demonstrated in *I. fumosorosea* at concentrations of 100 and 500 ppm. While this may significantly accelerate the infection and development of insect mycoses, this response may

also represent the manifestation of the defense mechanism of these microorganisms. According to Pusztahelyi *et al*. [38], NAG is a high molecular-weight hydrolytic lysosomal enzyme, which breaks chemical bonds of glycosides and amino sugars that form structural components in many tissues. NAG is necessary for the degradation and disposal of various parts of the cell, including the cell membranes. The degradation of insect cuticles may also be accelerated by elevated lipases activity. Lipases, in addition to increasing the degree of adhesion of fungal spores to insect cuticles, result in the hydrolysis of lipid compounds and phosphate esters, which leads to disturbances in the permeability of biological membranes [39,40]. Hence, the increased lipase and esterase activities demonstrated in *B. brongniartii* and *M. robertsii* that are in contact with Sn^{2+} ions at 1 ppm in this study may have an important and practical application. This aspect of EPF activity also permits the involvement of the proteases in the disease process in insects. There are numerous reports that show that proteolytic activity determines the pathogenicity of EPF [41,42]. EPF proteases appear to be less sensitive to contact with Sn^{2+} ions, as evidenced by their increased production by *B. bassiana*, *B. brongniartii* and *M. robertsii* at Sn^{2+} ion concentrations between 1-500 ppm and by *I. fumosorosea* at Sn^{2+} ion concentrations of 100 and 500 ppm. It is tempting to speculate that the activation of proteases may permit the increased hydrolysis of insect cuticle proteins even at toxic levels of Sn^{2+} ions. The amino acids released by this process may be used by the EPF as nutrients essential for their development [41,42]. In this context, the overproduction of hydrolases by EPF may have an ambiguous effect. On the one hand, this reaction may be considered a factor that leads to the increased pathogenicity of the insect pathogens. On the other hand, this may lead to an increase in mycelial survival in toxic conditions, in this case when the mycelia are in contact with Sn^{2+} ions. The diverse and ambiguous reactions of the EPF to the presence of inorganic Sn in the present study indicates the importance of broadening this study to other microorganisms. Microorganism contact with inorganic Sn^{2+}

ions in the environment is toxic but it can also lead to reactions in the microorganisms that could strengthen their beneficial role in the ecosystem.

In conclusion, in this report EPF that were tested exhibited a sensitivity to Sn^{2+} ions. The presence of these ions modified the development of the EPF and their biochemical activity. Sn^{2+} ions have fungistatic activity and could be used to restrict their growth in the environment as well as to influence the fungal communities in contaminated soils. Stimulation of the synthesis of extracellular enzymes, including NAG as well as some proteases and lipases, due to the action of Sn^{+2} ions translates directly into strengthening the role of these microorganisms in their pathogenesis of insects, and so the effectiveness of causing mycoses in plant pests. This study suggests that the use of EPF in biological plant protection in practice may differ from that detected in controlled conditions; this is due to the multifaceted and complex impact of metal ions that are present in the environment on the microorganisms.

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Author's contributions: ŁŁ and KMZ designed and conducted the experiments and analyzed the data, CT isolated EPF and AB analyzed data. All authors contributed to the preparation of the manuscript.

Tab. 1. The enzymatic activity of entomopathogenic fungi. Enzyme activity was determined with the API-ZYM test (C – Control; Sn_1 – Sn concentration 1 ppm; Sn_2 – Sn concentration 100 ppm; Sn_3 – Sn concentration 500 ppm)

	Enzyme	*B. bassiana*				*B. brongniartii*				*I. fumosorosea*				*M. robertsii*			
		C	Sn_1	Sn_2	Sn_3	C	Sn_1	Sn_2	Sn_3	C	Sn_1	Sn_2	Sn3	C	Sn_1	Sn_2	Sn_3
2	Alkaline phosphatase	2	1	1	1	3	4	3	3	1	1	1	1	1	2	2	1
3	Esterase (C4)	1	1	0	2	3	1	3	3	1	1	1	1	1	2	2	1
4	Esterase lipase (C8)	2	1	1	1	1	3	1	1	1	1	1	1	1	2	1	1
5	Lipase (C14)	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	Leucine arylamidase	2	3	3	1	3	3	4	4	1	1	1	1	1	3	3	1
7	Valine arylamidase	1	1	1	1	2	2	3	3	1	1	1	1	1	3	3	1
8	Cystine arylamidase	1	1	1	1	2	1	2	2	1	1	2	2	1	1	1	1
9	Trypsin	1	1	1	1	1	1	2	2	1	1	1	1	1	2	1	1
10	Chymotrypsin	1	1	1	1	1	1	2	2	1	1	1	1	1	2	1	1
11	Acid phosphatase	1	1	3	2	3	4	4	4	2	2	2	1	1	5	5	1
12	Naphthol-AS-BI-phospohydrolase	2	3	1	2	4	4	5	5	3	1	4	4	1	5	5	1
13	α-galactosidase	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	4
14	β-galactosidase	2	2	1	1	3	4	5	5	2	1	2	2	1	2	2	3
15	β-glucuronidase	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	3
16	α-glucosidase	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1
17	β-glucosidase	1	3	3	1	1	2	3	3	2	1	1	1	3	4	4	3
18	N-acetyl-β-glucosoaminidase	2	3	2	1	2	4	4	4	1	1	4	4	4	5	5	3
19	α-mannosidase	1	2	2	1	2	3	4	4	1	1	1	1	1	2	2	1
20	α-fucosidase	0	0	0	0	1	2	4	4	1	1	2	2	1	2	3	1

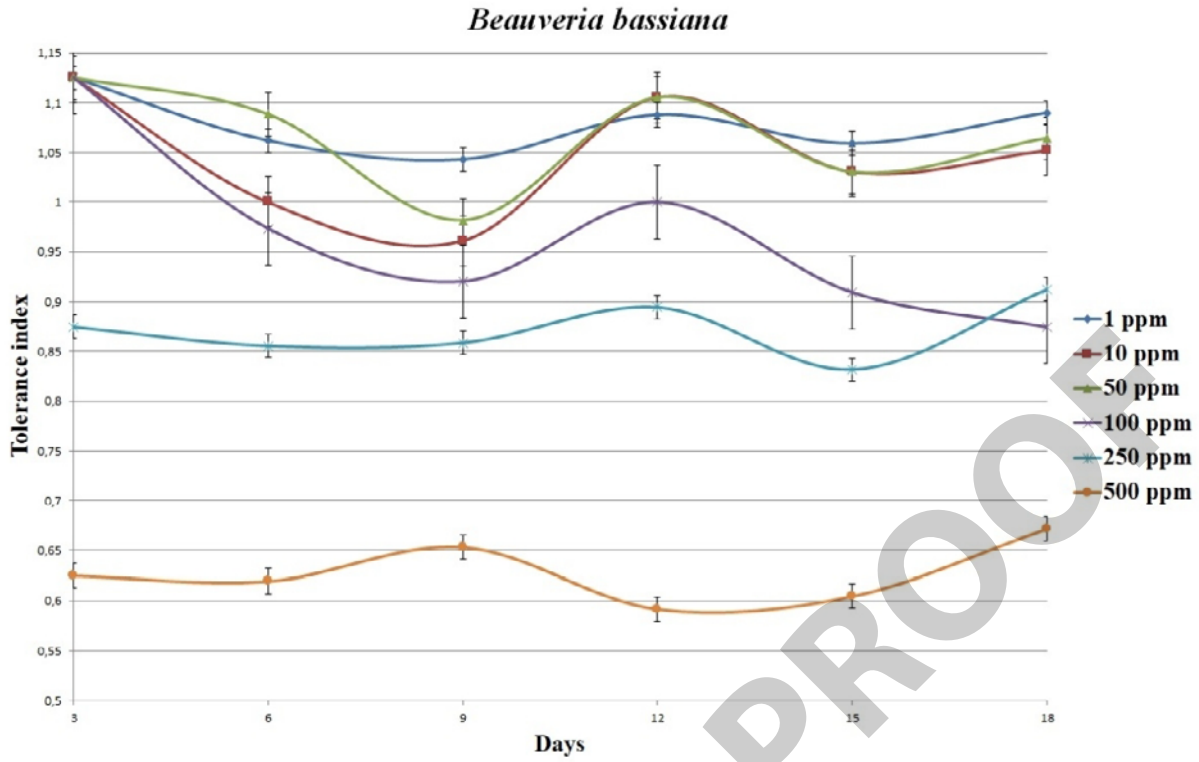

Fig. 1. Effect of an increasing Sn^{2+} concentration on the tolerance index of *Beauveria bassiana* over an 18 day incubation period.

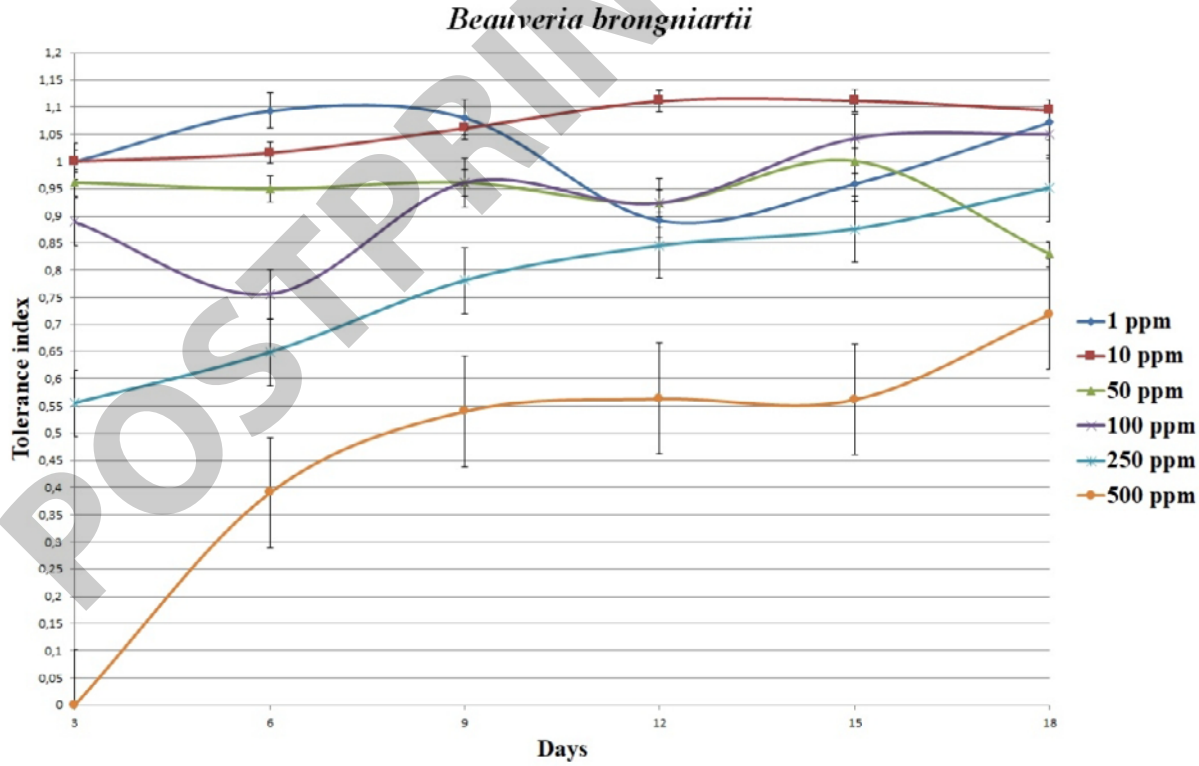

Fig. 2. Effect of an increasing Sn^{2+} concentration on the tolerance index of *Beauveria brongniartii* over an 18 day incubation period.

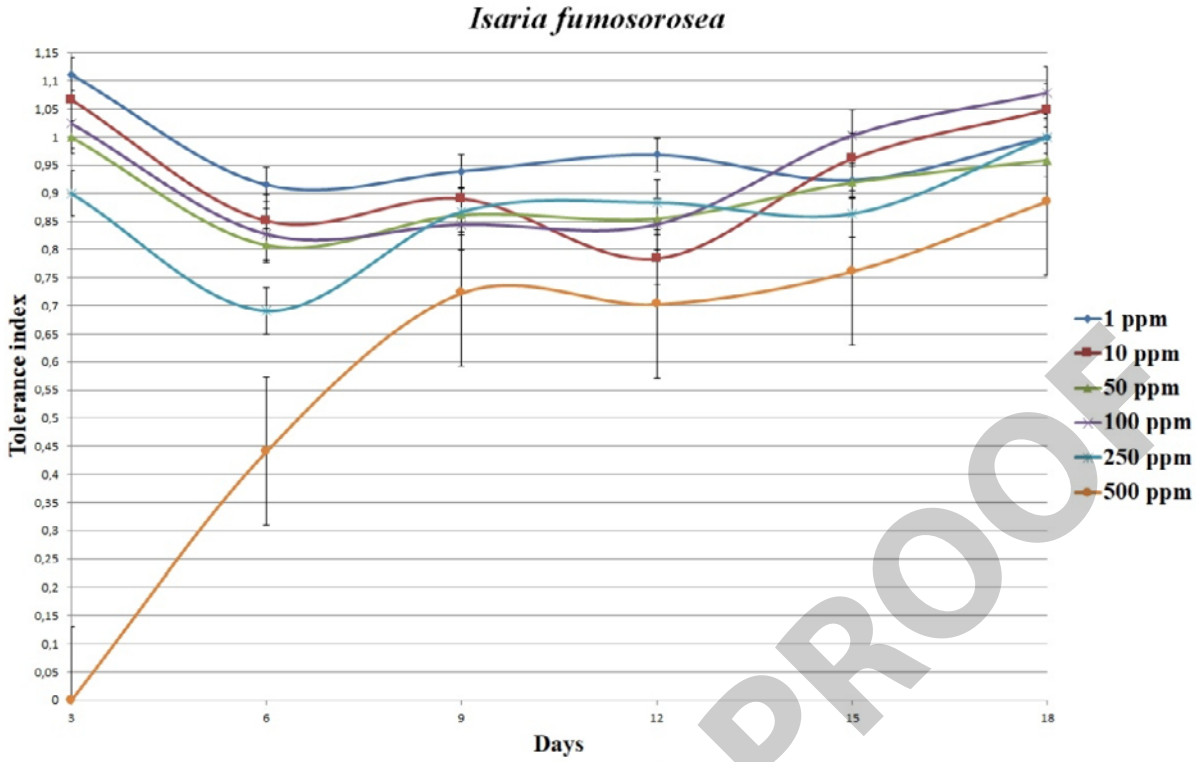

Fig. 3. Effect of an increasing Sn^{2+} concentration on the tolerance index of *Isaria fumosorosea* over an 18 day incubation period.

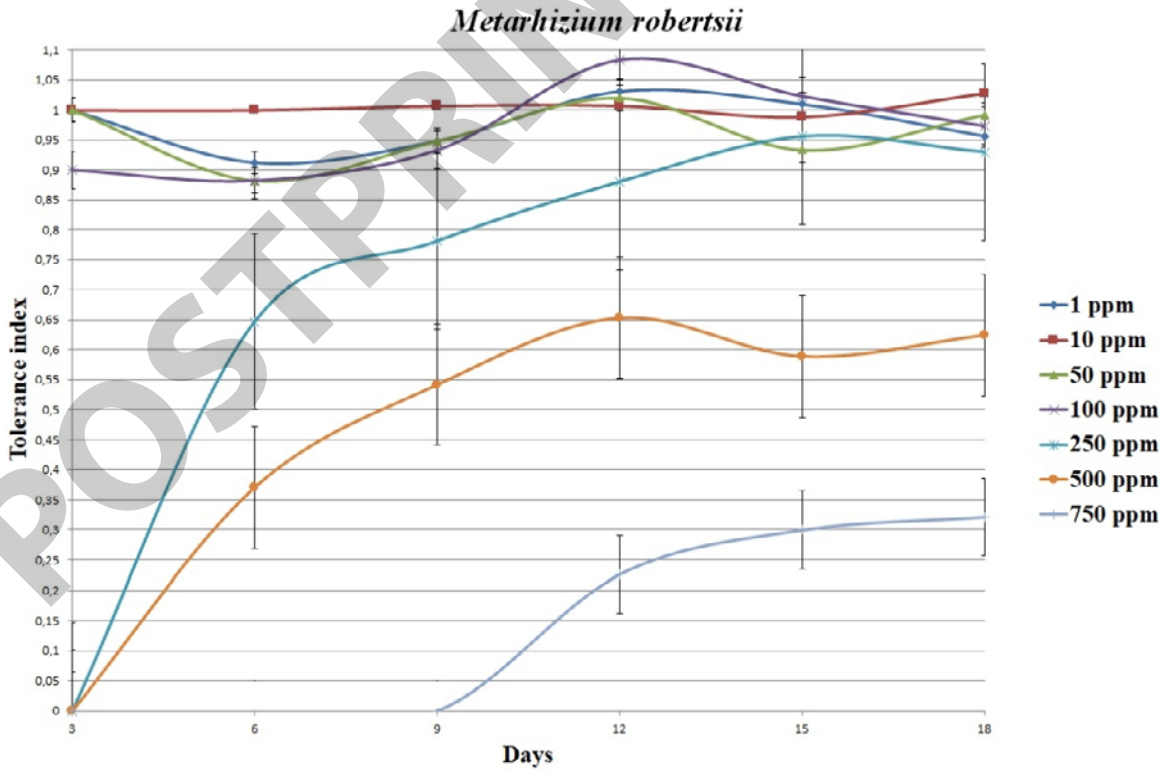

Fig. 4. Effect of an increasing Sn^{2+} concentration on the tolerance index of *Metarhizium robertsii* over an 18 day incubation period.