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Relationship between chemical properties of the soil and the occurrence of *Bacillus thuringiensis*

Relação entre as características químicas do solo e a ocorrência de *Bacillus thuringiensis*

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ABSTRACT

*The soil and other substrates such as mushroom compounds are the main sources of new **Bacillus thuringiensis** (Bt) isolates for Integrated Pest Management programs. This study describes the relationship between chemical properties of the soil (pH, OM, P^{3+}, K^{1+}, Ca^{2+}, Mg^{2+}, H^{1+}+Al^{3+}, B^{3+}, Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+}) and the occurrence of Bt in Brazil. A total of 1,197 bacterial colonies were obtained, being 512 of them identified as Bt. The Bt index (iBt), which is the relation between Bt colonies and bacterial counts ranged from 0.18 to 0.86. The iBt may be expressed with the formula: $iBt= -0.4 + 0.6Ca^{2+} + 0.07Cu^{2+} + 0.009Fe^{2+} – 0.53Mg^{2+} -0.12Mn^{2+} + 1.26Zn^{2+}$. A cluster of samples with fewer colonies and a high negative correlation (antagonism) between Mn^{2+} and Ca^{2+}; Mg^{2+} and Ca^{2+}; Mg^{2+} and Zn^{2+}; Mn^{2+} and Zn^{2+} and a high positive correlation (synergism) between Mn^{2+} and Mg^{2+}; Zn^{2+} and Ca^{2+} was observed. The relationship between these elements and their effect on the Bt presence are discussed.*

Key words: *Bt, chemical elements, entomopathogen, soil, biological control.*

RESUMO

*O solo e outros substratos, como restos vegetais são as principais fontes de obtenção de isolados de **Bacillus thuringiensis** (Bt) para programas de Manejo Integrado de Pragas. Este estudo descreve uma investigação sobre a relação entre algumas propriedades químicas do solo (pH, MO, P^{3+}, K^{1+}, Ca^{2+}, Mg^{2}+, H^{1+}+ Al^{3+}, B^{3+}, Cu^{2+}, Fe^{2+}, Mn^{2+} e Zn^{2+}) e a ocorrência de Bt em solos do Brasil. Entre 1197 colônias bacterianas, 512 foram identificados como Bt. O índice de Bt (iBt), que é a relação entre o número de colônias de Bt e de colônias bacterianas, variou de 0,18 a 0,86. Os dados obtidos mostraram que o iBt pode ser representado pelo iBt da fórmula = $-0,.4 + 0,6Ca^{2+} + 0,07Cu^{2+} + 0,009Fe^{2+} - 0,53Mg^{2+} - 0,12Mn^{2+} + 1,26Zn^{2+}$. Observou-se o agrupamento das amostras com poucas colônias; uma correlação negativa elevada (antagonismo) entre Mn^{2+} e Ca^{2+}, Mg^{2+} e Ca^{2+}, Mg^{2+} e Zn^{2+}, Mn^{2+} e Zn^{2+}; uma correlação positiva elevada (sinergismo) entre Mn^{2+} e Mg^{2+}, Zn^{2+} e Ca^{2+}. A relação entre esses elementos e o efeito dessa relação na presença de Bt são discutidos.*

Palavras-chave: *Bt, elementos químicos, entomopatógeno, solo, controle biológico.*

INTRODUCTION

Bacillus thuringiensis** (Bt)* is a widespread gram-positive spore-forming bacterium that produces toxic protein crystals named parasporal crystals. These inclusions are toxic to a wide variety of insects and many of them are commercially used against insect pests of Lepidoptera, Diptera and Coleoptera orders. *Bt* based biopesticides are the most successful ones and they represent approximately 80-90% of biological pest control agents worldwide (GLARE & O´CALLAGHAM, 2000; HABIB & ANDRADE, 1998). The serotype ***Bt kurstaki HD-1 became the basis for products that were competitive with chemical insecticides in performance and cost, and before long all of the *Bt* companies that produced *Bt* were

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producing this variety. It remains by far the greatest commercial success of microbial control (LORD, 2005).

The entomopathogen obtaintion, usually by the method called 'isolation', is the first and one of the most important steps for biopesticide formulation (ALVES et al., 1998). The soil and other substrates such as mushroom compounds are the main sources of new *Bt* for Integrated Pest Management programs.

Researches have been pointing the importance of *Bt* isolation from the soil (MARTIN & TRAVERS, 1989; CHILCOTT & WIGLEY, 1993; BERNHARD et al., 1997; HOSSAIN et al., 1997; DIAS et al., 1999; PINTO & FIUZA, 2003; URIBE et al., 2003). However, the influence of soil characteristics on *Bt* presence is poorly studied and this can affect the methods to isolate this bacterium. This research aimed to study the relationships between the chemical characteristics of some Brazilian soils and the presence of *Bt*.

MATERIAL AND METHODS

The research was carried out in the Laboratory of Pathology and Microbial Control of Insects, Department of Entomology, Phytopathology and Zoology of the 'Escola Superior de Agricultura Luiz de Queiroz' (ESALQ/USP), in Piracicaba, São Paulo State, Brazil. Brazil. Data were obtained from eight soil samples of the municipalities of Itapeva and Capão Bonito, São Paulo State. The soil samples analyzed (RAIJ et al., 2001) in the Department of Soil and Plant Nutrition of ESALQ/USP. The parameters evaluated were pH (CaCl), OM (organic matter), P^{3+}, K^{1+}, Ca^{2+}, Mg^{2+}, H^{1+}+Al^{3+}, B^{3+}, Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} (Table 1).

The method to isolate *Bt* was adapted from the World Health Organization protocol (WHO, 1985) with 1 g of soil dissolved in 10mL saline solution (0.006 mM $FeSO_4.7H_2O$; 0.01mM $CaCO_3.7H_2O$; 0.08mM $MgSO_4.7H_2O$; 0.07mM $MnSO_4.7H_2O$; 0.006mM $ZnSO_4.7H_2O$; pH 7.0) and shaken during 24 hours at 180rpm. One mL of this suspension was diluted 10 and 100 times. One mL was taken from this last suspension and submitted to a thermal shock (80° C for 12min) to eliminate undesirable microorganisms, e.g. fungi and protozoa. A total of 100µL of this suspension was transferred to plastic Petri dishes with Usual Medium (DE BARJAC & LECADET, 1976) for bacterial growth. Plates were incubated in a BOD chamber at 28ºC during 48 hours and the growth in each plate observed with a Colonies Counter model EC 550 A (Phoenix). Four replications were used per sampling (total of 32 plastic Petri dishes). A small portion of each colony was transferred to plastic tubes with 5mL Usual Medium and 5µL of Penicilliun G (JUNG et al., 1998). This material was shaken during forty-eight hours and examined under light microscopy (1,000 X) to verify the presence of parasporal body (crystal) that allows to differentiate ***B. thuringiensis*** from ***B. cereus*** (10) when the i*Bt* (*Bt* index = number of *Bt* colonies divided by the total ofbacterial colonies) was obtained. The data were submitted to a Cluster analyses, followed by the multivariate variance analyses and multiple regression (Stepwise) (SILVEIRA NETO, 1986).

RESULTS AND DISCUSSION

The counts of bacterial colonies and those of *Bt* colonies (Table 2) showed through the *Bt* index (i*Bt*) the success of this bacteria and that the soil is a good source of *Bt*. A total of 1197 bacterial colonies was obtained being 512 identified as *Bt* due to the presence of parasporal body. The i*Bt* ranged from 0.18 to 0.86 with an average of 0.42 compared to 0 to 0.2;

Table 1 - Soil chemical characteristics correlated with the presence of ***Bacillus thuringiensis*** *.

Sample [(1)]	pH	O.M.	P^{3+}	K^{1+}	Ca^{2+}	Mg^{2+}	H^{1+}+Al^{3+}	B^{3+}	Cu^{2+}	Fe^{2+}	Mn^{2+}	Zn^{2+}
01	4.1	27	7	2.7	13	4	58	0.28	2.9	123	70	1.5
02	3.9	32	6	0.7	1	1	109	0.23	1.1	83	1.2	0.2
03	4.2	29	6	2.6	16	6	47	0.38	3.8	43	60.8	0.8
04	3.7	31	5	0.8	1	1	88	0.30	1.3	43	0.4	0.2
05	3.9	32	6	0.9	1	1	98	0.29	0.8	47	1.0	0.2
06	3.9	29	6	0.7	1	1	72	0.29	0.5	50	0.4	0.1
07	3.7	23	5	1.6	1	1	80	0.41	0.4	47	0.4	0.2
08	4.0	21	5	0.5	2	2	58	0.24	0.4	45	0.6	0.2

*Date provided by Soil Department of ESALQ/USP obtained according to RAIJ et al. (2001). [1]Samples 01, 02 and 03: Campina Farm (Itapeva-SP); Samples 04, 05 and 06: Valinhos Farm (Capão Bonito, São Paulo State, Brazil); Samples 07 and 08: São Roque Farm (Capão Bonito, São Paulo State, Brazil).

Table 2 - Number of ***Bacillus thuringiensis*** (*Bt*) colonies and bacterian colonies and in the soil samples. The i*Bt* expresses the relation between these two types of colonies. Σ = Sum. X = Medium.

Samples	Number of *Bt* colonies (1)	Number of bacterial colonies (2)	i*Bt* (1/2)
01	58	183	0.31
02	26	138	0.18
03	57	110	0.52
04	58	150	0.38
05	102	118	0.86
06	51	188	0.27
07	56	139	0.40
08	104	171	0.60
	Σ = 512	Σ = 1,197	x = 0.42

0.02 to 0.85; 0.2 to 0.5 and 0.38 to 0.85 for DIAS et al. (1999) and HOSSAIN et al. (1997) and 0.04 to 0.16 in 144 samples analyzed with a similar method for *Bt* isolation from several Brazilian regions and from 0.09 to 0.07 for the southern Brazil (SILVA et al., 2002).

The higher values of i*Bt* than that obtained in SILVA et al. (2002) can be due to the fact that soil samples were shacked before the isolation of *Bt*. This increases the number of *Bt* isolates because this bacteria is released from the colloidal fraction of the soil. This method may explain why World Health Organization method is the most effective for Brazilian edaphic and climate conditions (SILVA et al. 2002). Another method proposed to isolate *Bt* comprised cultivation in liquid medium (JOHNSON & BISHOP 1996) but it was more expensive than others (OHBA & AIZAWA, 1986; DONOVAN et al., 1988; CAROZZI et al., 1991; MEADOWS et al., 1992). This new method was efficient but not in Brazilian conditions (SILVA et al. 2002). The higher number of *Bt* isolates was associated to the occurrence of insects where the soil samples were taken. This increases *Bt* dispersion and multiplication (HOSSAIN et al., 1997) but *Bt* was also found in mountains where insects are rare. Thus, abiotic factors, such as wind and rain, can favor the dispersion in microorganisms in the environment.

The great abundance of *Bt* isolates in Brazil may be due to the occurrence of higher number of insect pests or not pest than in other agricultural countries, which may recycle this entomopathogenic bacterium. Viable spores of *Bt* can survive from three to 16 months in the soil. For this reason, *Bt* recycling either by infected larvae or by growth using soil nutrients (SEKIJIMA et al., 1997).

A cluster of *Bt* colonies was observed with high negative correlation (the presence of these elements together inhibit *Bt* presence) between Mn^{2+} and Ca^{2+}; Mg^{2+} and Ca^{2+}; Mg^{2+} and Zn^{2+}; Mn^{2+} and Zn^{2+} and high positive correlation (the presence of these elements together favors *Bt* presence) between Mn^{2+} and Mg^{2+}; Zn^{2+} and Ca^{2+}. The *Bt* colonies may be expressed by the $iBt = -0.4 + 0.6Ca^{2+} + 0.07Cu^{2+} + 0.009Fe^{2+} - 0.53Mg^{2+} - 0.12Mn^{2+} + 1.26Zn^{2+}$. These negative and positive correlations were significant, but further studies are necessary to allow a satisfactory biological explanation for these results. The relationship between soil parameters and *Bt* presence showed that Cu^{2+} possibly contributes for resistant spores formation by environmental stress or that it inhibits growth of antagonist microorganisms (HOSSAIN et al., 1997). Another element, Ca^{2+}, is necessary for thermal stability of *Bt* spores, cellular development and δ-endotoxin production by this bacteria (DULMAGE & RHODES 1973; SIKDAR et al., 1991). The low concentrations (up to 0.5mg.dm^{-3}) of Zn^{2+} contributed to DNA and RNA synthesis of *Bt*. Concentrations from 0.5 to 50mg.dm^{-3} of Zn^{2+} destroyed proteins, avoided cellular development and the bacterial development was completely inhibited when the concentration of this element reached 60mg.dm^{-3} (YAO et al., 2002a). These results corroborate those of the present study, because all samples except the first one had Zn^{2+} with concentration about 0.5mg.dm^{-3} (Table 1). This explains the positive relationship between this element and the *Bt* presence (YAO et al., 2002a). The effect of Mn^{2+} on *Bt* growth showed that high concentrations (80 to 160 mg.dm^{-3}) of this element was important for the pathogen, but an opposite effect was observed at low concentrations of this element (below 80 mg.dm^{-3}) (YAO et al., 2002b). Negative interactions between the presence of Mn^{2+} and *Bt* was observed with all soil samples presenting values below 80 mg.dm^{-3}. This is important because an adequate quantity of this element is essential to start *Bt* sporulation (DULMAGE & RHODES, 1973).

The Mg concentrations were above the value considered the minimum (0.3 g.dm^{-3} of $MgSO_4$) for optimum production of ***Bt israelensis*** toxins (SIKDAR et al., 1991). The negative correlation between Mg^{2+} with Ca^{2+} and Zn^{2+} reduced the *Bt* occurrence in the samples. These authors determined that the minimum amount of Fe^{2+} (0.0025mg.dm^{-3}) is necessary for *Bt* toxin production. This agrees with the data obtained.

Some organic salts had concentrations too higher to be considered ideal for *Bt* (potassium, magnesium, phosphorous, sulfur and others) which are essential for microorganism growth (DULMAGE & RHODES, 1973). The presence of other elements may negatively influence the occurrence of this bacterium

in the soil such as found in the present study for Mn^{2+} and Mg^{2+}. It seems that each element has limited impact to benefit or harm *Bt* growth (YAO et al., 2002a; 2002b).

Soil microorganisms may be responsible for losses on the *Bt* δ–endotoxin activity which are in some intensity responsible for the inactivation of Bt spores (BENZ, 1987) but the presence of this bacterium was not affected by soil pH (HOSSAIN et al., 1997).

CONCLUSION

The researchers have to consider the chemical properties of the soil as an important factor that can affect the *Bt* isolation from soil samples, because some elements can contribute or reduce the occurrence of this microorganism.

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