Scientific Bulletin. Series F. Biotechnologies, Vol. XVII, 2013
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

IN VITRO STUDY ON THE INTERACTION BETWEEN *BACILLUS THURINGIENSIS* AND CHEMICAL PESTICIDES USED FOR CORN CROP PROTECTION

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Abstract

Interactions between the entomopathogenic bacteria Bacillus thuringiensis and chemical pesticides used for corn crop protection is one of the most important factors that influence the effectiveness of the entomopathogenic microorganism. Bacterial biopreparates based on B. thuringiensis could be used along with chemical pesticides. The effect of chemical ingredients on bacteria viability is mandatory and it should be conducted first. Interactions between the entomopathogenic bacteria B. thuringiensis and chemical pesticides occurs when chemicals and bacterial biopreparates are applied simultaneously or mixed together. Selectivity on some biological parameters of B. thuringiensis was tested by making a mixture of B. thuringiensis with chemical pesticides. Different concentrations of chemical pesticides were mixed with sporulated and vegetative bacterial cultures. The effects of chemical plant protection products on sporulation and vegetative growth of B. thuringiensis were monitored. The paper presents the results of experiments aimed at determining the influence of some chemical pesticides used for corn crop protection on the B. thuringiensis multiplication and sporulation.

Keywords: *Bacillus thuringiensis, chemical pesticides, corn crop.*

INTRODUCTION

Bacillus thuringiensis is an endospore-forming Gram-positive bacterium of economic importance due to its entomopathogenic capability and has been used as a safe microbial insecticide for over 50 years for caterpillars′ pest control. The insecticidal action of *B. thuringiensis* is attributed to protein crystals produced by the bacterium. *B. thuringiensis* based insecticides are popular with organic farmers because they are considered 'natural insecticides'. They differ from most conventional insecticides because they are toxic to only a small range of related insects (Hellmich, 2012). This is because specific pH levels, enzymes, and midgut receptors are required to activate and bind a given Cry toxin to midgut cells, which leads to pore formation in the insect's intestine and death (Federici 2002). Modern technology involves *B. thuringiensis* gene responsible for the production of the insecticidal protein incorporation into the maize genome for the corn borer control. Although *B. thuringiensis* based insecticides are an important tool for maize growers, they cannot completely replace the chemical control methods. That is why the bacterial entomopathogenic insecticides should be especially compatible with traditional pest management practices. Chemical plant protection products is one of the most important factors that influence the effectiveness of entomopathogic bacteria *B. thuringiensis* used in corn pest control.

Mixtures of bacterial biopreparates based on *B. thuringiensis* with different chemicals are possible and can be used in practice. The effect of chemicals on active substance of bacterial bioproducts must be checked.

The interaction between entomopathogenic bacteria and pesticides can occur in the following ways:

(1) Corn pests and diseases form a rich complex of species that cause damages in our country. Simultaneous control of diseases and pests could be made using plant protection chemicals. It is also very important to maintain a natural biological balance, to protect the environment and the useful insects. The systems of integrated protection in agriculture use chemical pesticides to control (a) Diseases: seedling damping-off (*Pythium* sp.), foot rot (*Fusarium* spp.), head smut (*Sorosporium*

holci-sorghi), corn smut (*Ustilago maydis*), (b) Pests: maize leaf weevil (*Tanymecus dilatecollis*), European corn borer (*Ostrinia nubilalis*), wireworms (*Agriotes* sp.), locusts and (c) Annual and perennial monocotyledonous and dicotyledonous weeds (*Amaranthus* sp., *Chenopodium* sp., *Sinapis* sp., *Capsella* sp., *Thlaspi* sp., *Cirsium* sp., *Hibiscus* sp., *Xanthium* sp., *Abutilon* sp., *Raphanus* sp., *Solanum* sp., *Polygonum convolvulus*, *Setaria* sp., *Echinochloa* sp., *Digitaria* sp., *Convolvulus arvense*, *Calystegia sepium*).

(2) Application of chemical pesticides in the mixture or simultaneously with bacterial bioproducts, depending on the evolution of insect pest, in order to make treatments more profitable.

(3) Application of some pesticides containing bacterial preparation in order to obtain adequate efficacy.

MATERIALS AND METHODS

T4 strain of *B.thuringiensis* var. *thuringiensis,* from Research-Development Institute for Plant Protection collection of micro-organisms, was used for this experiment.

The following working method was used in order to identify the selectivity of plant protection products against entomopathogenic bacteria. Bacterial culture was grown in corn extract agar media which was mixed with each of the chemicals in the following three concentrations: the recommended concentration for use (c.u.), ½ (1/2c.u.) and ¼ (1/4c.u.) of recommended concentration for use.

Test mixtures were sown in Petri dishes, which were incubated at 28^0C for 72 hours.

Different chemicals from fungicides, insecticides and herbicides groups were tested (Table 1).

Table 1. Plant protection chemicals tested in combination with bacterial culture

FUNGICIDES			
Chemical group	**Product (s.a.)**	**Target organism**	**Dose (conc.)**
Dithiocarbamates and thiuram derivatives	ROYAL FLO 42 S (thiram 480g/l)	*Pythium* spp. *Fusarium* spp.	3,0 l / t seeds
Triazoles and imidazoles	VITAVAX 200 FF (carboxina 200g/l+thiram 200 g/l	*Pythium* spp. *Fusarium* spp.	2,5 l / t seeds
INSECTICIDES			
Synthetic pyrethroids	SIGNAL (cypermethrin 300 g/l)	*Agriotes* spp.	2,0 l/t seeds
Various	ACTARA 25 WG (thiamethoxam 25%)	*Tanymecus dilaticolis*	0,100 kg/ha
	GAUCHO 600 FS (imidacloprid 600 g/l)	*Tanymecus dilaticolis* *Agriotes* spp.	6,0-8,0 l pc/t seeds
	COSMOS 250 FS (fipronil 250 g/l)	*Agriotes* spp.	5,0 l/t seeds
	CRUISER 350 FS (thiamethoxam 350 g/l)	*Tanymecus dilaticolis* *Agriotes* spp.	1,2 µl/one seed
HERBICIDES			
Aminofosfats	DOMINATOR (glyphosate 360 g/l)	Annual and perennial weeds	4,0 l/ha
	ROUNDUP (Glyphosate isopropylamine salt 360 g/l)	Monocotyledonous and dicotyledonous weeds, annual and perennial (+*Sorgum halepense* from rhizomes)	4 l/ha (mixed with 100-150 l water/ha)
Picoline derivatives	CERLIT (fluroxypyr 250 g/l)	*Convolvulus arvensis* *Calystegia sepium*	1,0 – 2,0 l/ha
Sulfonylureas	MISTRAL 4 SC (nicosulfuron 40g/l)	*Sorghum halepense*	1,5 l/ha
	TITUS 25 DF (rimsulfuron)	Monocotyledonous weeds, annual and perennial – including *S.halepense* from seeds and rhizomes- and some annual dicotyledonous weeds	60 g/ha
Isoxazoles	CALLISTO 480 SC (mesotrine 480 g/l)	Annual weeds	0,350 l/ha
Mixtures	CALLAM (tritosulfuron 12,5% + dicamba 60%)	Annual and perennial dicotyledonous weeds	0,4 + 1,0 l/ha

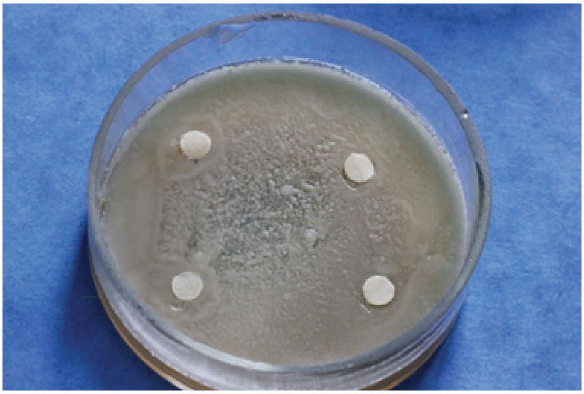

Figure 1. Filter paper discs with sterilized distilled water on *B. thuringiensis* bacterial lawn (corn extract agar media)

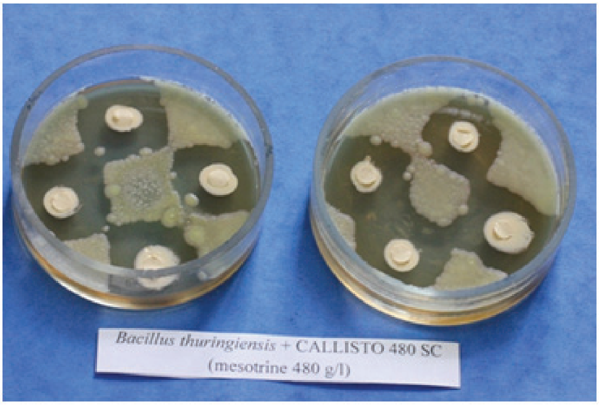

Figure 2. Effect of mesotrine on halo formation and inhibition of the growth of *Bacillus thuringiensis* on corn extract agar media

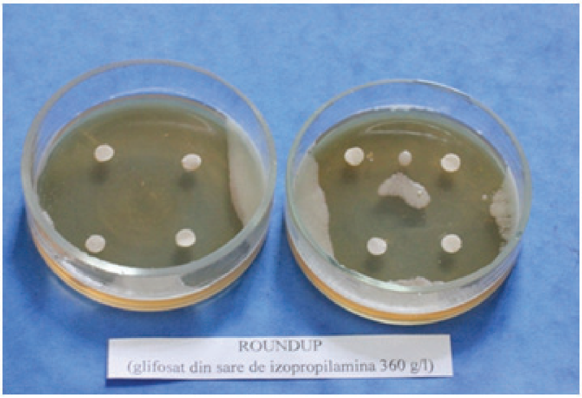

Figure 3. Effect of glyphosate isopropylamine salt on halo formation and inhibition of the growth of *Bacillus thuringiensis* on corn extract agar media

In order to make a better observation on selectivity of plant protection products against *B. thuringiensis* strain, a common working method was used for some of the variants.

Bacteria was inoculated in Petri dishes on corn extract agar media, followed by the placement of 4 paper discs with the evaluated insecticide in 4 points of the dish. In the control treatment, four filter paper discs with sterilized distilled water replaced the insecticide (Figure 1). The data was analyzed on the 4th and 7th day after the treatment application. Each Petri dish was analyzed for the absence or presence of the bacterial growth inhibition halo (Figures 2, 3)

The tested chemicals are commonly used in maize crop protection. Observations followed *B. thuringiensis* colony diameter treated with pesticides variants, compared with untreated control variants.

RESULTS AND DISCUSSIONS

Results on the effect of chemicals on spore germination and vegetative multiplication of *B. thuringiensis* are presented in Tables 2, 3 and 4. Bacterial growth on agar media was noted as follows: + + + = very good growth, + + = good growth, + = weak growth.

An overall analysis of the data presented reveals that the tested products fall into one of these 3 categories: (a) products with high selectivity towards *B. thuringiensis* (b) products with average (good) selectivity and (c) products with low selectivity (bacteriostatic). This analysis refers to how bacterial growth and spore germination of *B. thuringiensis* have been influenced by contact with plant protection chemicals

Bacteriostatic properties of some chemicals influenced spore germination and vegetative multiplication in varying proportions. This effect was revealed through the partial inhibition of vegetative and sporal multiplication at high concentrations of the chemical. Bacterial cultures mixed with recommended concentration for use of chemicals belonging to this category and inoculated on agar media, developed colonies with a diameter from two to eight times lower than the bacterial colonies of untreated control variant.

By decreasing the concentration of the chemical in the mixture experiments, development of bacterial cultures was registered normal parameters. The size of bacterial colonies was close or equal to those of the control variant.

Microscopic analysis showed no changes in vegetative or sporulated bacterial cells.

The following issues were observed for the groups of chemicals: (a) fungicides have generally manifested stronger inhibitory effect on bacterial spores; (b) insecticides manifested, in general, high degree of selectivity against *B. thuringiensis* and (c) herbicides manifested the lowest degree of selectivity.

There was an almost complete inhibition of bacterial growth, both in vegetative and sporulated culture when mixed concentration of DOMINATOR and ROUNDUP herbicides dose corresponded to recommended concentration for use.

Partial inhibitory effect was maintained when the concentration was reduced by two or four times.

Recent studies aimed at rice crops protection, revealed the compatibility between insecticides (thiamethoxam, labdacyhalothrin, malathion and fipronil) and *B. thuringiensis* strains (*B. thuringiensis* subsp. *dendrolimu*s, *B. thuringiensis* var. *kurstaki*, *B. thuringiensis* var. thuringiensis and *B. thuringiensis* subsp. entomocidus) interaction. However, at a 10^1 concentration, ten times higher than the recommended concentration, some insecticides presented inhibitory effect in the bacterial development. The insecticide malathion inhibited the development of six out of seven evaluated *B. thuringiensis* strains at the higher concentration (Pinto et al., 2012).

Batista-Filho et al. (2001) and Almeida et al. (2003) reported compatibility between *B. thuringiensis* bacterial growth and thiamethoxam insecticide.

Silva et al. (2008) reported resistant *B. thuringiensis* var. *kurstaki* colonies expressing inhibition in the presence of some herbicides.

Field toxicity studies have shown that when chemical insecticides manifest in vitro toxicity against *B. thuringiensis*, this does not suggest necessarily high field toxicity (Alves et al., 1998).

It is recommended, though, chemical insecticides be used in the advised doses when using *B. thuringiensis*-based products.

Table 2. Influence of some fungicides on *Bacillus thuringiensis* Experimental mixture tested Bacterial lawn /Fungicide concentration

Variants	Chemical substance +	*Bacillus thuringiensis*	c.u.	½ c.u.	¼ c.u.
I (a)	ROYAL FLO 42 S	vegetative	+++	+++	+++
I (b)		sporulated	+++	+++	+++
II (a)	VITAVAX 200 FF	vegetative	+++	+++	+++
II (b)		sporulated	+++	+++	+++

Table 3. Influence of some insecticides on *Bacillus thuringiensis* Experimental mixture tested Bacterial lawn /Insecticide concentration

Variants	Chemical substance +	*Bacillus thuringiensis*	c.u.	½ c.u.	¼ c.u.
I (a)	SIGNAL	vegetative	+++	+++	+++
I (b)		sporulated	+++	+++	+++
II (a)	ACTARA 25 WG	vegetative	++	+++	+++
II (b)		sporulated	++	++	+++
III (a)	GAUCHO 600 FS	vegetative	+++	+++	+++
III (b)		sporulated	+++	+++	+++

Table 4. Influence of some herbicides on *Bacillus thuringiensis* Experimental mixture tested Bacterial lawn / Herbicide concentration

Variants	Chemical substance +	*Bacillus thuringiensis*	c.u.	½ c.u.	¼ c.u.
I (a)	DOMINATOR	vegetative	-	+	++
I (b)		sporulated	-	+	++
II (a)	ROUNDUP	vegetative	-	+	++
II (b)		sporulated	-	+	++
III (a)	TITUS 25 DF	vegetative	+++	+++	+++
III (b)		sporulated	+++	+++	+++
IV (a)	CALLISTO 480 SC	vegetative	+++	+++	+++
IV (b)		sporulated	+++	+++	+++
V (a)	CALLAM	vegetative	+++	+++	+++
V (b)		sporulated	+++	+++	+++

CONCLUSIONS

The overall effect of the chemicals on *B. thuringiensis* efficiency is difficult to assess in field conditions.

On one hand, we consider the average concentration of pesticides with which bacteria come into contact is in a lesser amount than the one tested in the laboratory. Occasionally, those which can be applied directly to the soil could exceed the normal dose. On the other hand, growth of *B. thuringiensis* on agar media, with optimal conditions, makes it more tolerant for chemicals compared to bacteria released into nature where it has to face less favorable conditions, competition with antagonists etc. Therefore, experimental variants which showed good selectivity of chemicals against bacteria *B. thuringiensis* in controlled laboratory conditions should be tested under field conditions too.

Based on the data presented, the tested chemicals fit within these degrees of selectivity in relation to *B. thuringiensis.*

Table 5. Selectivity of chemicals against *B. thuringiensis*

	High selectivity	Good selectivity	Low selectivity
Fungicides	ROYAL FLO 42 S VITAVAX 200 FF	-	-
Insecticides	SIGNAL GAUCHO 600 FS CRUISER	ACTARA 25 WG	-
Herbicides	MISTRAL 4 SC TITUS 25 DF CALLISTO 480 SC CALLAM.	ESTERON 60 ATRANEX 80 WP ROMANEX 500 SC ALANEX 48 EC	DOMINATOR ROUNDUP

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