SOME CHEMICAL ADDITIVES TO INCREASE THE ACTIVITY SPECTRUM OF BACILLUS THURINGIENSIS VAR. KURSTAKI (DIPEL 2X) AGAINST THE RICE MOTH CORCYRA CEPHALONICA

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SUMMARY : Some chemical additives have been adopted to increase the potency of D-endotoxin of Bacillus thuringiensis var. Kurstaki (Dipel 2X) against the rice moth Corcyra cephalonica. Procedures were based on the incorporation of some selected essentially non toxic and low cost compounds with different modes of action with the endotoxin to increase its activity. Among the compounds tested were some inorganic salts, nitrogenous compounds, protein solubilizing agents, sugars, some plant powders and some plant extracts.

Key Words : Bacillus thuringiensis var. Kurstaki, corcyra cephalonica.

INTRODUCTION

Few reports are available concerning the possible interaction between the bacterial endotoxin with chemicals and their impact on the host susceptibility to the endotoxin effect. Charles *et al.* (3) reported an increase in the larval mortality of the gypsy moth *Porthetria* dispar upon incorporation of boric acid with the endotoxin of *B. thuringiensis* ssp. *thuringiensis* sprayed on leaves of lettuce plants. Smirnoff (14) found that chitinase enhanced the septicemia caused by *B. thuringiensis* when applied against *Choristoneura fumiferana.* Salama *et al.* (10) reported that the potency of *B. thuringiensis* spp. *entomocidus* and aizawai HD-133 against *S. littoralis* can be increased by modifying the conditions prevailing in the insect midgut and through incorporation of alkaline compounds, proteolytic activators and some mildly toxic compounds. The present investigations aimed to select some chemicals with respect to their possible synergistic interactions with the bacterial S-endotoxin preparation of the commercial product Dipel 2X (Abbott) using larvae of *Corcyra cephalonica* as the target insect. The compound chosen must be essentially non-toxic to man or animal, possesses no harmful effect on plants at the tested concentration, biodegradable and commonly available at low price. The chemical tested induced representatives of the inorganic salts, nitrogenous compounds including amino acids, protein solubilizing agents, sugars, some plant powders and some plant extracts.

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MATERIALS AND METHODS

A standard laboratory culture of the rice moth Corcyra cephalonica was maintained on a diet of minced wheat grains in glass jars 2 liter capacity at 28 ± 2°C. Ten days old larvae were used throughout the experimental work. A control fed on natural diet (without toxin) was used to determine the larval mortality when fed on a diet containing different concentrations of each of the tested chemicals. The concentration of each additive at which larval mortality was within normal limits (up to 20%) was selected for testing in combination with the endotoxin of the commercial product Dipel 2X at a constant level of (500 mg/mg of diet). The

percentage of larval mortality on a diet treated with chemical additive alone or with endotoxin alone and a combination of the additive with endotoxin was recorded. Data on mortalities were corrected using Abbott's formula (1) and the cotoxicity factor calculated according to Monsour *et al.* (6).

$$\text{Cotoxicity factor} = \frac{\text{Observed mortality - expected mortality}}{\text{Expected mortality}} \times 100$$

The bioassay was replicated 5 times using 20 larvae for each replicate. All tests were made under room condition of 28 ± 2°C.

The compounds tested in the present study were as follows:

1. Inorganic salts :

Including sodium carbonate, calcium carbonate, ammonium sulphate, ammonium dibasic phosphate, potassium monobasic phosphate, potassium dibasic phosphate, potassium sulphate, copper sulphate and zinc sulphate.

2. Nitrogenous compounds :

Including DL-alanine, L-arginine, L-leucine, Proline and serine.

3. Protein solubilizing agents :

Urea and ethylene diaminetetra acetic acid (EDTA).

4. Sugars :

Sucrose, galactose, lactose and D-mannose.

5. Plant powders :

Pomegranate peel (*Punica granatum,* family puiniaceae), guava leaves (*Psidium guawava,* family Myrataceae), eucalyptus leaves (*Eucalyptus globulus* L., family Myrataceae), fenugreen seeds (*Trigonella faenum gracum* L., family Leguminoseae) and lupin seeds (*Lupinus termis forks,* family Leguminoseae).

The plant materials were dried in shade and ground into line powder in a Moulinex Blender for 4-5 min. The powders were then tested for the toxicity as admixtures in 0.5 g of powders per 100 g of sterilized minced wheat grains diet.

6. Plant extracts :

Different solvent extracts of two plant powders were tested for their effects.

a) Method of Extraction

i) Matter extract

The powder of fenugreek and lupin extracted with organic solvents such as petroleum ether, acetone and methanol. A known weight (200 g) of powdered seeds of each plant was subjected to extraction using one solvent for 30 hours or longer. The petroleum ether extract was filtered through anhydrous sodium sulphate and the collected extract was then evaporated at room temperature. The same procedure was followed for acetone and methanol.

The extracts of different host plants were bio-assayed for their effect. To 5 ml of each remaining paste and solvent, 95 ml distilled water were added in 250 ml glass beakers and one drop of tween 80 and the mixture was well agitated to get suspensions of 5% extract (5 ml extract/95 ml water) of the tested samples containing 5% plant extract.

Different extracts were tested for their toxic effect. Ten ml of the extract suspension were mixed with 100 g diet. The treated diet was placed in plastic cups and replicated 5 times. Untreated wheat, with solvent only and without solvents, served as control.

Twenty ten days old larvae were introduced in each cup and used to evaluate the larval mortality in larvae fed on the treated diet. In another series of experiments, the percentage of larval mortality was recorded when the larvae were fed on a diet treated with 5% plant extract combined with 500 μg/ml *B. thuringiensis* suspension. Data on mortalities were corrected using Abott's formula (1) and the cotoxicity factor was calculated according to Mansour *et al.* (6). The bioassay was replicated 5 times and

Table 1: Effect of inorganic salts on the activity of *B. thuringiensis* var. *Kurstaki* (Dipel 2X) (500 μg/g).

Salt tested with Dipel 2X None (endotoxin)	Percent larval mortality		Cotoxi. factor	Type of inter.
	Salt alone	Salt + endotoxin		
only		62.34		
Ammonuim sulphate (1%)	6.1	68.3	-0.2	AD
Ammonium dibasic phosphate (1%)	17.1	54.9	-30.9	AN
Calcium carbonate (0.5%)	25.0	84.0	-3.8	AD
Copper sulphate (1%)	14.4	51.1	-33.4	AN
Potassium monobasic phospate (1%)	16.4	61.2	-22.3	AN
Potassium dibasic phosphate (1%)	16.4	61.2	-22.3	AN
Potassim sulphate (1%)	3.0	46.7	-28.5	AN
Sodium carbonate (0.5%)	22.0	42.2	-49.9	AN
Zinc sulphate (0.1%)	20.0	71.1	-13.6	AD

AD= Additive, AN= Antagonism, P= Potentiative

twenty ten days old larvae were used in each replicate.

RESULTS

1. Inorganic salts

Nine inorganic salts of ammonium, calcium, potassium, sodium, copper and zinc the concentrations of 1% or 0.5% except zinc concentration of which was 0.1%. The results show the effect of these salts as remarkable additives or antagonists to the endotoxin activity of *Bacillus thuringiensis* var. *Kurstaki* (Dipel 2X) vs. *Corcyra cephalonica.*

The data given in Table 1 show that the incorporation of ammonium sulphate (1%), calcium carbonate (5%) and zinc sulphate (0.1%) caused an additive effect by increasing the low initial effect of the endotoxin. On the other hand, the other salts showed an antagonistic effect as they reduced the toxic effect of the endotoxin.

Table 2: Effect of amino acids on the activity of (Dipel 2X) (500 μg/g).

Compound tested with Dipel 2X	Percent larval mortality		Cotoxicity factor	Type of interaction
	Compound alone	Compound + endotoxin		
None (endotoxin only)		62.34		
DL-Alanine	26.9	100.00	+12.1	AD
L-Arginine	60.1	100.00	-18.3	AD
L-Leucine	2.9	100.00	+53.4	P
Proline	32.6	46.3	-51.2	AN
Serine	36.0	55.4	-43.7	AN

AD= Additive, AN= Antagonism, P= Potentiative

Table 3: Effect of protein solubilizing agents on the activity of (Dipel 2X) (500 μg/g).

Compound tested with Dipel 2X	Percent larval mortality		Cotoxicity factor	Type of interaction
	Compound alone	Compound + endotoxin		
None (endotoxin only)		62.34		
Urea	26.1	71.5	-19.07	AD
Ethylene diamine tetra acetic acid (EDTA)	25.3	78.95	-9.88	AD

AD= Additive, AN= Antagonism, P= Potentiative

Table 4: Effect of sugars on the activity of (Dipel 2X) (500 μg/g).

Compound tested with Dipel 2X	Percent larval mortality		Cotoxicity factor	Type of interaction
	Compound alone	Compound + endotoxin		
None (endotoxin only)		62.34		
Galactose	20.0	73.10	-11.2	AD
Lactose	9.7	96.8	+34.4	P
D-Mannose	1.2	51.5	-18.9	AD
Succrose	25.0	72.0	-17.6	AD

AD= Additive, AN= Antagonism, P= Potentiative

2. Nitrogenous compounds

Five amino acids, D-L alanine, L-arginine, L-leucine, proline and serine were tested at a concentration of 0.05%.

The data given in Table 2 show that the incorporation of D-L-alanine and L-arginine with the bacteria exert an additive effect in terms of increasing the low initial effect of the endotoxin. In addition, L-leucine makes a remarkable potentiation of S-endotoxin while proline and serine gives a reserve effect as they reduce the toxic effect of the endotoxin.

3. Protein solubilizing agents

Among protein solubilizing agents, ethylene diamine tetra acetic acid (EDTA) and urea, were tested and incorporated into the larval diet at a concentration of 0.5% containing *B. thuringiensis.*

It appears from data in Table 3 that EDTA and urea showed additive effects as they increased the initial effect of the endotoxin.

4. Sugars

The possible role of some sugars as adjuvants was also tested.

The data in Table 4 show that the incorporation of galactose, lactose, D-mannose and succrose at a concentration of 5%, into the larval diet obtaining *B. thuringiensis* caused a remarkable potentiation and additive effect on the endotoxin activity. Lactose showed a notable potentiation of D-endotoxin, galactose, D-mannose and succrose showed an additive effect which appeared to increase the effect of the endotoxin.

Table 5: Effect of plant powders on the activity of Dipel 2X.

Compound tested with Dipel 2X	Percent larval mortality		Cotoxicity factor	Type of interaction
	Compound alone	Compound + endotoxin		
None (endotoxin only)		56.4		
Guava leaves powder	4.2	53.5	-11.7	AD
Eucalyptus leaves powder	25.5	63.0	-23.1	AN
Pomegranate peel powder	12.1	80.7	+17.8	AD
Lupin see powder	42.1	93.4	-5.17	AD
Fenugreek seed	15.78	79.47	+10.09	AD

AD= Additive, AN= Antagonism, P= Potentiative

5. Plants powders

Deterrents induced by some plant powders were tested. The posers of pomegranate peel (*Punica granatum, family Puniaceae*), guava leaves (*Psidium guajava, family Myrataceae*), eucalyptus leaves (*Eucalyptus globulus L., family Myrataceae*), fenugreek seeds (*Trigonella faenum gracum, family Lefiminoseae*) and lupin seeds (*Lupinus termis forks,* family Leguminoseae) were incorporated at a concentration into the larval diet containing the endotoxin at 500 μg/g.

The data given in Table 5 show that a relatively high larval mortality (42.1%) of *Corcyra cephalonica* was obtained when fed on a diet of the plant additive powder. A moderate mortality (25.5%) resulted in case of eucalyptus leaves powder, while normal mortalities occurred with guava leaves, pomegranate peel and fenugreek seeds powder. The incorporation of these powders into larval diet containing the endotoxin at 500 μg/g diet showed that pomegranate peel, guava leaves, lupin seeds and fenugreek seeds powder gave an additive effect which appeared to increase the initial effect of the endotoxin. However, eucalyptus leaf powder showed an antagonistic effect as it reduced the toxic effect of the endotoxin.

6. Plants extracts

The effect of different solvent extracts of two seed powders fenugreek and lupin were tested for their insecticidal activity against *Corcyra cephalonica* larvae.

The data given in Table 6 show that the acetone extract of fenugreek and lupin caused a high larval mortality when incorporated with endotoxin, being 77.2% and 82.6%, respectively. The petroleum ether extract of fenugreek and lupin gave 69.6% larval mortality when incorporated with the endotoxin, while the methanol extract of fenugreek and lupin gave a larval mortality of 66% and 73.9%, respectively.

The tested extracts could be arranged according to their efficiency as follows :

Fenugreek seed powder

Acetone > petroleum ether > methanol,

Lupin seed powder

Acetone > methanol > petroleum ether.

It appears that the incorporation of these extracts at a concentration of 5% into the larval diet containing *B. thuringiensis* had a remarkable additive effect on the endotoxin activity.

Generally, the tolerance of the insect to the action of *Bacillus thuringiensis* must be taken into consideration.

DISCUSSION

Among the tested inorganic salts, sodium and calcium carbonate caused a remarkable increase in the larval mortality and this may possibly be due to the alkaline nature of these salts, which increases the endotoxin breakdown and releases more toxic fragments from the crystals (7). In case of zinc sulphate, the mode of action may be correlated with its effect on the proteolytic enzyme present in the insects midgut. It is assumed that the bacteria may be the synergist of these salts and not vice versa. This may be the case when the bacteria slow down the de-toxication of the salts.

Guerra (4) found that the amino acids may interfere with the normal physiological processes causing a biochemical change in the haemolymph composition. Under the present conditions, the amino acids may easily penetrate through the midgut cells previously affected by B*acillus thuringiensis* into the haemolymph thus affecting their susceptibility to the endotoxin.

The mode of action of protein solubilizing agents may be due to their effect in reducing the disulphide linkage in protein molecules which has a significant role in preventing

Table 6: Effect of some plant extracts on the activity of *B. thuringiensis* var. *Kurstaki* (Dipel 2X) (500 μg/g).

Solvent extracts	Percent larval mortality				Cotoxicity factor		Type of interaction	
	Fenugreek Extract alone	Extract + endotoxin	Lupin Extract alone	Extract + endotoxin	Fenugreek	Lupin	Fenugreek	Lupin
None (endotoxin only)				62.34				
Methanol	0.8	66.0	19.6	73.9	4.53	-9.8	AD	AD
Pet. Ether	20.7	69.6	8.7	69.6	-16.18	-2.02	AD	AD
Acetone	20.7	77.2	25.0	82.6	-7.03	-5.43	AD	AD

AD= Additive, AN= Antagonism, P= Potentiative

dissolution of the crystalline endotoxins of *B. thuringiensis* subspecies. This is due to the reduction of these bonds in endotoxin molecule to sulphydral groups thus increasing endotoxin solubility in the insects gut and leading to a high larval mortality (7,11).

Sugars affect the feeding response of the larvae, as they act as gustatory stimulatants affecting the feeding response of different insect pests and have shown promise in increasing the effectiveness of the microbial diseases (5,12).

The tested plants incorporated into the diet induced an obvious retardation in the development and longevity of the tested larvae as compared to untreated ones. This result agrees with that given by Salama and Sharaby (9), who mentioned that the incorporation of guava leaves powder (1%) into the diet induced a significant retardation in the larval and pupal development of *Spodoptera littoralis* and a drastic reduction of the percentage of adult emergence. Generally, the effect of such materials may be attributed to one or more of the following : fumigants, contact or stomach poisoning effect, when feeding the insects on admixed grains. Moreover, the potentiating effect of these plant powders may be due to the presence of some chemical groups in their structure, for example pomegranate peel contains alkaloids, tannins, mono and triterpenes (13).

Osman *et al.* (8) also reported that the leaves of guava and eucalyptus contain flavonoids, B-sitosterol and volatile oils. The petroleum ether of the two tested seeds mainly contain sterols, unsaturated sterols, triterpenes and traces of saponins and tannins. Acetone extracts contain mainly flavonoids, tannins, traces of glycosides and alkaloids. Methanol extracts contain flavonoid, alkaloids, glycosides and traces of saponins and tannins (only in the case of fenugreek) (2).

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