EFFECT OF SOME CHEMICAL ADDITIVES ON THE POTENCY OF *BACILLUS THURINGIENSIS* AGAINST THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS*

MOHAMED, E. M.[1], HANAN. F. ABDEL – HAFEZ[2] AND MAHASEN A. ABDL- AZIZ[1]

1. *Sids Agric. Res. Station, Plant Protection Res. Institute , ARC, Giza*
2. *Cotton Pesticides Bioassay Dept, Plant Protection Res. Institute, ARC, Giza*

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Abstract

Field experiments were conducted to investigate the effect of inorganic salts, zinc sulphate ($ZnSO_4$), Calcium carbonate ($Ca\ CO_3$) and Calcium oxide (Ca O) on potentiation of *Bacillus thuringiensis* (*Bt)* formulations against the 2nd instar larvae of *Spodoptera littoralis*. Physico-chemical properties, suspensibility, surface tension and pH were also studied under field dilution rate (200 L water/ fed.).

The results indicated that the tested inorganic salts decreased surface tension and increased PH value of candidate *B.t.* The initial and residual activity of the tested *B.t,* Dipel 2X, Protecto and Agerin can be markedly increased by using $ZnSO_4$, $CaCO_3$ and Ca O at 0.1% in combination with their full recommended rates. Zinc sulphate was the most effective salt in increasing both initial and residual effect of Dipel 2X, Protecto and Agerin,. Zinc sulphate ($ZnSO_4$) also increased the residual half life value (RL50) of Diple 2X, Protecto and Agerin from 4.5, 3.6 and 3.2 days to 6.25, 7.0 and 7.0 days, respectively. The population of *Spodoptera littoralis* larvae were also reduced after 7 days after application.

The addition of such salts decrease surface tension of *Bt* preparations, then increase their wettability and spreading on treated plant leaves, and increase their initial and residual activity. Also, to change pH of the gut, being more alkaline and thus enhancing the endotoxin breakdown and release of toxic fragments.

INTRODUCTION

The hazards of pesticides and development of resistance to chemical insecticides in the cotton leafworm, *Spodoptera littoralis,* necessitates the use of safe and effective insect pathogens such as *Bacillus thuringiensis.*

B. thuringiensis (*Bt*) preparations are prime candidates for use in Integrated Pest Management Programme (IPM). They have high pathogenicity for target pests. Safe for most non-target organisms, and have good integration with other pest control methods. It is Known that most *Bt* formulations have a very short residual activity, in cotton fields in Egypt has been found that the persistence of spores of *B.t* showed an obvious reduction after few days of weathering and decoy in its viability was progressively correlated with the time of exposure in the field, because it has a

very short effective residual life. The pathogen is not mobile and cannot escape under the unfavorable conditions. (Moar *et al.*, 1986 and Morris *et al.,* 1996).

In order to increase *B.t* potency, the conditions prevailing insect midgut must be modified. This might be achieved by using alkaline compounds. These compounds must be non-toxic to man or animal, possess no harmful effect on plants, biodegradable and commonly available at low price such as inorganic salts (Salama, *et al.*, 1989).

Therefore, the present study was carried out to investigate the role of some alkaline additives (inorganic salts) to increase the potency of B.t and enhance the residual life against the cotton leafworm *S. littoralis* in cotton field.

MATERIALS AND METHODS

1-insect: Sufficient egg-masses of cotton leafworm, *Spodoptera littoralis* were collected from Sids Agricultural Res. Station, Beni-Sueif Governorate during 2009 cotton season. Larvae were reared under laboratory conditions at 25± 2Cº and 60± 5% R.H. The 2nd instar larvae were used in all field experiments.

2- *B.t* Formulations

a- Dipel 2x : 6.4% W.P. based on *Bacillus thuringiensis* subsp. Kurstaki (32,000 IU/mg). Produced by Abbott laboratories, North Chicago. U.S.A.

b- Protecto: 9.4% W.P. based on *Bacillus thuringiensis* is subsp. Kurstaki, it contains 9.4% Lepidopteran active toxin produced by the Plant Protection Res. Inst. A.R.C.

c- Agerin: 6.5% WP. based on *Bacillus thuringiensis* subsp *Serovara egypti*. Produced by Agricultural Genetic Engineering Res. Inst., ARC.

3-Chemical additives

(a) Calcium Carbonate ($CaCO_3$).

(b) Calcium Oxide (CaO).

(C) Zinc Sulphate (Zn SO_4) all additives were supplied by El-Gomhouria chemical Co.

4-Physico-Chemical properties: were studied for the tested B.t formulations alone and their combinations with chemical additives. PH value was measured using PH- meters, Surface tension was determined using Findley method (1941) While, Suspensibility test was carried out according to WHO Specifications (1979).

5-Field and semi field experiments: Field trials were performed in cotton fields in Sids Agric. Res. Station, Beni-Suief Governorate during June 2009. To evaluate the initial and residual activity of the candidate *B.t* alone and their

mixtures with inorganic salts, the biocides, Diple 2x, Protecto and Agerin were applied at their recommended rates 200 gm, 300gm and 250gm / fed., respectively and their combinations with 0.1% $ZnSo_4$, $CaCO_3$ and CaO. The treatments were sprayed on cotton plants variety 80 using a solo motor under field dilution rate (200 L water/ fed.) For semi field evaluation samples of treated cotton leaves were randomized collected from each treatment at different intervals (Zero, 2 and 7 days) after application. Such leaves were transferred to the laboratory and offered to 2 nd instar larvae of *S. littorlis*.

Five replicates (ten larvae / treatment) were Fed on treated leaves for 48 hrs. then the alive larvae fed on untreated Cotton leaves until pupation. Other five replicates were fed on untreated cotton leaves as check. The treated larvae were examined daily to determine the mortality percentages. Accumulative larvae mortality was recorded and corrected using Abbott's formula (1925). Pupation and adult emergence percentage of treated and untreated larvae were also determined. For field evaluation *S. littoralis* infestations were evaluated by examining 100 randomly selected plants. The average number of *S. littoralis* larvae present in plants in each plot was recorded after three time intervals (zero, 2 and 7 days) after application. Larval reduction percentages were calculated using Hendrson & Telton (1955) equation as follows:

Reduction % = 1 – {A/B x C/D} x100

A and B = No. of larvae in check before and after application, respectively.

C and D = Number of larvae in the treatment after and before application.

RESULTS AND DISCUSSION

1-Physico-chemical properties

The physico-chemical properties (suspensibility, surface tension and pH) of Dipel2x, Protecto and Agerin either alone or in mixtures with chemical additivies were measured under filed dilution rate (200L-water/fed.). Data in Table (1) indicated that all the tested additives were compatible with candidate *B.t*, they gave good suspension without any precipitation was observed at the bottom of cylinder after half an hour. Suspenibility were considered as a limiting factor for the successful combination and would be an important guide for the physical compatibility of the mixed materials, good suspension shouldn't give any precipitation after half an hour (WHO specification, 1979). Also all the tested inorganic salts decreased surface tension when cambined with Dipel 2x, Protecto and Agerin. This reduction would increase their wettability and spreading on treated plant leavs, then increase residual

activity (Osipow, 1964). Acidity levels of the tested *B.t.* formulations either alone or in combination with tested chemical additvies also are shown in table (1). Zinc sulphate, calcium carbonate and calcium oxide increased pH value of Dipel 2x from 6.6 to 7.95, 8.40 and 10,00 respectively, Also the tested inorganic salts increase pH value of Protecto from 6.80 to 8.01, 8.20 and 9.99, respectively. Finaly the pH value of Agerin was increased from 6.12 to 7.75, 8.23 and 9.88, respectively. The activity of delta-endotoxin of B.t. preparation increased as alkalinity increased from 8 to 10 and then, declined rapidly at pH >10 (Gringorten *et al.*, 1992 and Venugopal *et al.*, 1992). Girgis, (2003) reported that pH, conuctivity and salinity for the combinations of biocides with potassium and calcium carbonate increased both toxicity and residual effect against *Phthorimaea operculella.*

Table 1. Effect of adding chemical additives on the physico-chemical properties of Dipel 2x, Protecto and Agerin under field dilution rates.

Treatments	Rate / fed. (gm)	Precipitation (ml)	Surface tension (dyne / cm)	pH
Dipel 2X alone	200	0.0	50.99	6.60
+ ZnSO4	200+ 0.1%	0.0	47.08	7.95
+CaCO3	+ 0.1%	0.0	48.96	8..40
+ Ca O	+ 0.1%	0.0	47.08	10.00
Protecto alone	300	0.0	61.2	6.80
+ ZnSO4	300 + 0.1%	0.0	43.71	8.01
+CaCO3	+ 0.1%	0.0	53.22	8.20
+ CaO	+ 0.1%	0.0	47.04	9.99
Agerin alone	250	0.0	58.29	6.12
+ ZnSO4	250+0.1%	0.0	50.99	7.75
+CaCO3	+0.1%	0.0	45.33	8.23
+ CaO	+0.1%	0.0	47.08	9.88

2- The initlial and residual effects of the tested B.t formulations and their combinations with chemical additives under field conditions

2.1. Semi-field experiment

Persistence of *B. thuringiensis* is very short in cotton field of Egypt, because it is affected by exposure to ultraviolet radiation. Biochemical approches were adopted in an attempt to increase potentiation the pathogen against Lepidopterus insects. These approaches were based on the incorporation of some selected non-toxic chemical compounds such as inorganic salts (Hafez, *et al.*, 1998).

The initial and residual effect of *B.t.* alone and their mixters with 0.1% $ZnSo_4$, $CaCO_3$ and CaO at three time intervales (Zero, 2 and 7 days) post application against 2nd instar larvae of *S. littoralls* are shown in Table (2). Data revealed that $ZnSO_4$ at 0.1% increased mortalities when combined with Dipel 2x, Protecto and Agerin at full recommended dose, it caused 100% larval mortalities at the initial time interval (zero-day) as compared to *B.t.* alone 93.6, 80.85 and 72.34%, respectively. Also $CaCo_3$ increased the initial kill when used at 0.1% with tested *B.t*. It increased mortalieties from 93.6, 80.85 and 72.34% to 100, 95.74 and 97.87% for Dipel 2x, Protecto and Agerin, respectively. Finaly CaO increased mortalities when mixed at 0.1% with tested biocides at zero time, it produced 97.87, 91.49 and 82.98%. The obtained RL_{50} values are tabulated in Table (2). All the tested chemical additives increased RL_{50}'s of Dipel 2x, Protecto and Agerin. $ZnSO_4$ increased RL_{50} values of Dipel 2x, Protecto and Agerin from 4.5, 3.6 and 3.2 days to 6.25, 7.0 and 7.0 days, respectively. Also $CaCO_3$ increased RL_{50} values of such *B.t* agents Diple 2x , Protecto and Agerin from 4.5, 3.6 and 3.2 days to 6.0, 6.0 and 5.8 days, respectively. On the other hand CaO was the lowest one in increasing RL_{50} values of Dipel 2x, protecto and Agerin, it increased the RL_{50} from 4.5, 3.6 and 3.2 days to 5.7, 5.7 and 4.8 days Pupation and adult emergance percentages were also determined . The results in Table (2) indicated that all the tested inorganic salts decreased both pupation and adult emergance percentages at initial and residual time intervales when such salts used at 0.1% with full recommended rate of Dipel 2x, protecto and Agerin comparing with the control which recorded pupation and emergence rates of 98.0 and 96.0%, respectevily.

Table 2. Initial and residual activity of the tested *B.t* formulations and their mixtures with chemical additives against *S. littoralis* treated as 2nd instar larvae, under field conditions.

Treatments	Rate / fed (gm)	Accumulative larvae mortality after indicating time intervals days)									RL_{50} (days)
		Initial kill			Residual effect						
		Accumulative zero day			Accumulative 2 days			Accumulative 7 days			
		% Corrected Mortality	% Pupation	% Adult emergence	% Corrected Mortality	% Pupation	% Adult emergence	% Corrected Mortality	% Pupation	% Adult emergence	
Dipel 2X alone	200	93.6	6	2.0	36.96	58.0	50.0	28.57	70.0	68.0	4.5
+ ZnSO4	200+ 0.1%	100.0	0.0	0.0	63.04	34.0	20.0	48.88	50.0	44.0	6.25
+CaCO3	+ 0.1%	100.0	0.0	0.0	60.87	36.0	24.0	44.89	54.0	50.0	6.0
+ Ca O	+ 0.1%	97.87	2.0	0.0	54.35	42.0	34.0	36.73	62.0	52.0	5.7
Protecto alone	300	80.85	18.0	14.0	43.48	52.0	52.0	20.41	78.0	70.0	3.6
+ ZnSO4	300+ 0.1%	100.0	0.0	0.0	69.66	28.0	20.0	40.82	58.0	54	7.0
+CaCO3	+ 0.1%	95.74	4.0	0.0	67.39	30.0	22.0	38.78	60.0	56.0	6.0
+ Ca O	+ 0.1%	91.49	8.0	2.0	63.04	34.0	26.0	36.73	62.0	58.0	5.7
Agerin alone	250	72.34	26.0	20.0	30.43	64.0	60.0	20.41	78.0	70.0	3.2
+ ZnSO4	250+ 0.1%	100.0	0.0	0.0	67.39	30.0	22.0	48.98	50.0	40.0	7.0
+CaCO3	+ 0.1%	97.87	2.0	0.0	54.35	42.0	32.0	44.89	54.0	50.0	5.8
+ Ca O	+ 0.1%	82.98	16.0	6.0	36.96	58.0	42.0	36.73	62.0	60.0	4.8
Control	---	---	94.0	97.9	---	92.0	88.0	---	98.0	96.0	---

RL_{50}= Residual half life value

It could be concluded that the initial kill, residual effect and RL_{50} values of the tested B.t formulations markedly increased by using $ZnSo_4$, $CaCO_3$ and CaO at 0.1% in mixtures with full recommended rates of condidate *B.t* (Dipel 2x, Protecto and Agerin). Zinc sulphate was the most effective additive, it showed a remarkable effect in enhancing *B.t* potency.

The mode of action of this salt may be correleated to its effect on the proteolytic enzymes present in the insect midgut. Also, calcium salts such as calcium carbonate and calcium oxide, drastically enhanced the potency of *B.t.* This may be attributed to the addition of such salts will change pH of the gut, being more

alkaline and thus enhancing the endotoxin breakdown and release of toxic fragments (Salama, *et al.* 1989). Hefez *et al.* (1998) reported that inorganic salts such as Calcium oxide (CaO), Calcium Carbonate ($CaCO_3$), Zinc Sulphate ($ZnSO_4$) and Potassium Carbonate (K_2CO_3) at 0.1% potentiated the activity of the product, Dipel 2x against the corn borers, *Chilo agamemnon* and *Ostrinia nubilalis*. Also, Girgis (2003) and (2007) found that K_2Co_3 and $CaCO_3$ at 0.1% increase the initial, residual effect and RL_{50} values of some biopesticides combined at their full recommended doses against *Phthorimaea operculella* under laboratory and green house conditions.

2.2 Field experiment

The results of reduction percentage of *S. littoralis* population after three indicating time intervals are summarized in table (3) Data showed that all the tested chemical additives reduced the population of *S. littoralis* larvae after three indicating time intervals of application (zero, 2 and 7 days) when combined at 0.1% with their full recommended concentration of Dipel 2X, Protecto and Agerin. Zinc sulphate reduced the population of *S. littoralis* larvae after 7 days of application when mixed at 0.1% with the tested *B.t.* The average reduction was 64.44, 57.77 and 56.66 % for Dipel 2X, Protecto and Agerin, respectively. Also, $CaCO_3$ increased reduction percentage after 7 days of application from 46.66, 41.11 and 31.11 to 60.00, 60.66 and 53.33 %. Calcium oxid also increased reduction % from 46.66, 41.11 and 31.11 to 57.77, 50.00 and 48.89 %. Data clearly show that the highest potency was observed when $ZnSo_4$ was mixed at 0.1% with tested *B.t* formulation.

In conclusion, the results of field and semi field evaluation, clearly showed that the tested inorganic salts appeared promising in combination with candidate *B.t.* formulations as a result of increasing their initial and residual effect and reducing the population of *S. littoralis* larvae after three time intervals.

These results are in agreement with those mentioned by Salama, *et al* (1990). They reported that addition of 0.1% $CaCO_3$ and $ZnSO_4$ to Dipel 2x, greatly increased initial and residual effect and reduced larval populations of *S. littoralis* and led to a significant increase in yield in some vegetable crops. Wen *et al*. (1999) studied the effects of six inorganic chemical additives on the efficacy of *B. thuringiensis* against *Plutella xylostella*. They found that K_2CO_3 and $ZnSO_4$ were effective and caused a 1.075 and 1.1535 fold increase in *B.t* toxicity. Shalaby, *et al*. (2007) found that assayed treatments, bacterial biopesticides when combined with K_2CO_3 at 1%, produce 80% reduction in egg masses of *Sesamia cretica* in Maize fields.

Table 3. Effect of *B.t.* formulations and mixtures with chemical additives on the larvae of *S. littoralis* treated as 2nd instar larvae.

Treatment	Rate/Fed	% Reduction after indicating time interval (days) ± S.E			Average Reduction %
		Zero day	2 days	7 days	
Dipel 2X alone	200	73.33±2.7	60.00±4.7	6.67±2.7	46.66
+ ZnSO4	200+ 0.1%	83.33±2.7	73.33±2.7	36.66±2.7	64.44
+CaCO3	+ 0.1%	80.00±4.7	70.00±4.7	30.00±4.7	60.00
+ Ca O	+ 0.1%	80.00±4.7	66.66±4.7	26.66±2.7	57.77
Protecto alone	300	66.66±2.7	50.00±0.0	6.67±2.7	41.11
+ ZnSO4	300 + 0.1%	76.66±2.7	63.33±2.7	33.33±2.7	57.77
+CaCO3	+ 0.1%	73.33±2.7	66.67±2.7	40.00±4.7	60.00
+ CaO	+ 0.1%	66.66±4.5	60.00±0.0	23.33±2.7	50.00
Agerin alone	250	53.33±2.7	36.67±2.7	3.33±2.7	31.11
+ ZnSO4	250+0.1%	76.66±2.7	66.66±2.7	26.66±2.7	56.66
+CaCO3	+0.1%	73.33±2.7	63.33±2.7	23.33±2.7	53.33
+ CaO	+0.1%	70.00±4.7	60.00±4.7	16.67±2.7	48.89

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تأثير إضافة بعض الإضافات الكيميائية لزيادة الفاعلية والتأثير المتبقي لبعض مستحضرات المبيدات الحيوية البكتيرية ضد دودة ورق القطن

عصام محمد محمد[1] ، حنان فاروق عبد الحافظ[2] ، محاسن احمد عبد العزيز[1]

١. *محطة بحوث سدس – معهد بحوث وقاية النباتات – مركز البحوث الزراعية*
٢. *قسم بحوث اختبار مبيدات افات القطن –– معهد بحوث وقاية النباتات – مركز البحوث الزراعية*

أجريت التجارب الحقلية لدراسة تأثير المواد الإضافية الكيميائية غير السامة (الأملاح الغير عضوية) مثل كبريتات الزنك وكربونات الكالسيوم وأكسيد الكالسيوم لزيادة فعالية بعض المستحضرات البكتيرية ضد العمر اليرقى الثاني لدودة ورق القطن أيضاً تم دراسة تأثير هذه الأملاح فى تغير الخواص الطبيعية– الكيميائية للمستحضرات البكتيرية المختبرة بمفردها وكذلك مخاليطها مع الأملاح الغير عضوية تحت التخفيف الحقلي (200 لتر ماء/فدان) كاختبار التعلق– التوتر السطحي– درجة الحموضة)

أوضحت النتائج أن إضافة هذه الأملاح بتركيز 0.1% يؤدى إلى خفض التوتر السطحي وزيادة في درجة الحموضة للمستحضرات البكتيرية المختبرة.

أيضاً تؤدى هذه الأملاح إلى زيادة كلاً من الأثر الإبادى الفوري والباقي وزيادة فترة نصف العمر للمستحضرات البكتيرية المختبرة. وكانت كبريتات الزنك من أكفأ الأملاح الغير عضوية المختبرة فى زيادة كلاً من الأثر الفوري والباقي لكل من الدايبل 2 × و البروتكتو والأجرين حيث أدى إلى زيادة فترة نصف العمر لهذه المستحضرات من 4.5، 3.6، 3.2 يوم إلى 6.25، 7، 7 يوم على الترتيب. كذلك أدت إلى زيادة فى خفض تعداد يرقات دودة ورق القطن بعد 7 أيام من المعاملة.

ويعزى هذا إلى أن إضافة مثل هذه الأملاح تؤدى إلى خفض التوتر السطحي للمستحضرات المختبرة مما يؤدى الى زيادة التبلل والانتشار للمبيد الحيوي على أوراق النباتات المعاملة وعندئذ زيادة كلا من الأثر الفوري والباقي واستدامة هذه المركبات.

أيضا زيادة الأس الهيدروجينى في معدة الحشرات تجعلها أكثر قلوية ومن ثم زيادة فاعلية هذه المركبات الحيوية البكتيرية.