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Spray and dust applications of *Bacillus thuringiensis* Berliner and Lannate against *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) on soybean plants in Egypt

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Abstract

Bacillus thuringiensis var. *kurstaki* HD-1 (Dipel 2×), as a biological insecticide was tested against *Spodoptera littoralis* larvae infesting soybean plants either alone or combined with potassium carbonate as an adjuvant. The carbamate chemical insecticide, lannate, was used for comparison. The materials were applied either through spraying or dusting. Results showed that potassium carbonate enhanced and significantly increased the effect of Dipel. Both techniques are suitable for use in control application. The recommendation of using a combination of Dipel at 250 g/f and potassium carbonate (150 g/f) is advisable. This combination gave significant high larval reduction (96.86 % and 92.11 %) with an increase of 2.50 and 1.60 folds in yield after spraying and dust applications, respectively. The data also suggested that a combination of Dipel and potassium carbonate (Dipel + K₂CO₃) may be an effective component of the future *S. littoralis* IPM programs on soybeans.

1 Introduction

The protection of agricultural crops, man, and his domestic animals from damage by various pests and toxic residues of chemical insecticides remains a serious problem. Concerning health aspects, the residue analysis of Thiodicarb = Larvin (a new selective carbamate insecticide) showed that the amount detected on and in the soybean foliage was 2.64 ppm, 15 days after treatment (ALMAZ et al. 1986). Such foliage will not be suitable for feeding animals according to United States Environmental Protection Agency, whereas the temporary established tolerance was 0.1 ppm (ANONYMOUS 1982).

Therefore, the use of entomopathogens, particularly the bacterium, *Bacillus thuringiensis* in suppressing many lepidopterous pests has been studied as an alternative for reducing the use of chemical insecticides (ABUL-NASR and ABDALLAH 1970; ABDALLAH and ABUL-NASR, 1970; MORRIS 1980).

Efforts have been already made with the aim of developing the bacterium *B. thuringiensis* as a microbial control agent for the control of the Egyptian cotton leaf worm, *Spodoptera littoralis*, either alone (SALAMA et al. 1981a, 1983; SALAMA and FODA 1982), in a combination with chemical insecticides (SALAMA et al. 1984a), mixed with adjuvants or some chemical additives (SALAMA et al. 1984b), or combined with other forms of entomopathogens such as the nuclear polyhedrosis virus NPV (SALAMA et al. 1987).

SALAMA et al. (1984a) reported that the incorporation of 0.05 % potassium carbonate in the insect diet caused a marked increase in the potency of *B. thuringiensis* against *S. littoralis*. They stated that the economic implications of these results remain to be determined after application in the field, so as to overcome the problem of short persistence of *B. thuringiensis* under the adverse field conditions.

In the present study, field experiments were conducted to compare the effectiveness of

the biological insecticide *B. thuringiensis* var. *kurstaki* HD-1 (Dipel 2×) and the chemical insecticide, Lannate when applied as spray or dust for the control of the Egyptian cotton leafworm, *Spodoptera littoralis* on soybean plants.

2 Materials and methods

During 1988, at Beni-Suef governorate, Mancarish village, a series of field tests were conducted to assess the control of *S. littoralis* infestations on soybean following application of Dipel 2× singly or combined with potassium carbonate as an adjuvant (SALAMA et al. 1985, 1986) compared with the chemical insecticide, Lannate. Soybean, variety Clark was cultivated on 25 June, 1988, and plants received all the normal agricultural practices during the growing season. On August 2, an area of about 5 feddans was selected for field experiments. In this area, a high infestation level with *S. littoralis* was found.

In all tests, Dipel 2× (32,000 IU/mg of *B. thuringiensis* var. *kurstaki* HD-1) was applied at the rate of 250 and 125 g/f. Potassium carbonate was used at the rate of 150 g/f. The chemical insecticide Lannate was used at the recommended rate (300 g/f) for comparison. Treatments were replicated in plots each (40 m × 20 m) = 800 m² = plot. The tested materials were either applied as spray or dust. In spray application, molasses was added to aqueous suspension of Dipel 2× at the rate of 3 l/f. Knapsac sprayer was used for spraying (200 l/f). For dust application, the tested material was mixed with 25 kg wheat flour/f. The Elec Engine, GMD-503 L, Arimitsu Industry Co., Japan, was used for dusting application.

2.1 Effect on the larvae

The average number of *S. littoralis* larvae present on 10 plants in each plot was recorded just before treatments, 7 days and 10 days after treatments. Percentages of larval reduction (% L. R.) were calculated according to LEWIS and LYNCH (1978). At least 5 counts, 10 plants/each count were used for each treatment. Data were analysed by DUNCAN'S (1951) multiple range test.

2.2 Yield assessment

The study was extended to evaluate the effect of the two techniques on the crop yield. The yield was harvested on 25th October. The dry plants were removed from an area of 10 m × 10 m = 100 m² representing each replicate, seeds separated and the average weight per 100 m² was estimated and the yield was then calculated per feddan. This test was replicated 5 times/treatment. Analysis of variance of the data was also made using DUNCAN'S (1951) multiple range test.

3 Results

3.1 Foliar spraying technique

3.1.1 Effect on larvae

Pre-treatment counts as presented in table 1 indicate that the population of *S. littoralis* larvae ranged between 176.0 to 186.0/10 plants. After 7 days, all treatments caused a significant reduction in the larval population, as compared to the control. Comparisons between treatments show that Lannate was the best. The addition of potassium carbonate to Dipel significantly increased the potency of *B. thuringiensis* against the larvae than if it was used singly. Ten-days after spraying, the data clearly indicated that the effect of Lannate and a combination of Dipel at 250 g/f and potassium carbonate at 150 g/f were almost identical. There were significant differences in larval reduction after treatments with Dipel 2× at 250 or 125 g/f.

3.1.2 Effect on yield

The data presented in table 2 show that all treatments significantly increased the yield more than that of the control. The level of yield increase ranged between 1.78 to 2.62 folds. Comparisons between treatments showed that potassium carbonate (150 g/f) when added

Table 1. Effect of foliar spraying against *S. littoralis* infesting soybean plants at Beni-Suef governorate

Treatment	Dose/feddān (g)	¹ Mean larval count \pm S.E./10 plants Indicated days after treatment			% Larval reduction	
		Zero	7	10	7 days	10 days
Control	—	² 183.0 \pm 19.144a	181.75 \pm 9.653a	191.25 \pm 11.122a	—	—
Dipel 2x	250	178.0 \pm 17.073a	13.50 \pm 4.770c	8.50 \pm 1.414c	92.57	95.56
	125	186.0 \pm 7.628a	29.25 \pm 3.562b	19.50 \pm 1.479b	83.91	89.80
Dipel 2x + K ₂ CO ₃	250 + 150	176.0 \pm 9.165a	10.00 \pm 3.082c	2.25 \pm 0.829d	94.50	98.82
	150 + 150	180.0 \pm 12.227a	12.25 \pm 1.479c	6.00 \pm 1.871c	93.26	96.86
Lannate	300	185.0 \pm 12.748a	5.00 \pm 1.414d	3.75 \pm 1.090d	97.25	98.04

¹ Average of 5 counts, 10 plants/each count. — ² Means followed by the same letter are not significantly different at (P = 0.05) according to DUNCAN's multiple range test.

Table 3. Dust application of Dipel and Lannate against *S. littoralis* larvae infesting soybean plants at Beni-Suef governorate

Treatment	Dose/feddān (g)	¹ Mean larval count \pm S.E./10 plants Indicated days after treatment			% Larval reduction	
		Zero	7	10	7 days	10 days
Control	—	184.5 \pm 14.858a	174.0 \pm 16.477a	177.5 \pm 26.519a	—	—
Dipel 2x	250	177.8 \pm 17.570a	31.3 \pm 10.825bc	14.25 \pm 3.345c	82.01	91.97
	125	178.0 \pm 27.559a	35.5 \pm 5.500b	21.75 \pm 2.385b	79.60	87.75
Dipel 2x + K ₂ CO ₃	250 + 150	181.3 \pm 21.568a	22.0 \pm 5.874d	12.25 \pm 2.165c	87.36	93.10
	125 + 150	176.8 \pm 18.512a	28.0 \pm 5.339c	14.00 \pm 3.240c	83.91	92.11
Lannate	300	183.75 \pm 8.136a	15.0 \pm 1.707e	12.00 \pm 1.829c	91.38	93.24

¹ Means in a column followed by the same letter are not significantly different at (P = 0.05) according to DUNCAN's multiple range test.

to Dipel at the two dosages (250, 125 g/f) significantly ($P = 0.05$) increased the yield over that of Lannate or Dipel alone. Thus, it appears that the higher dose of Dipel 2 \times (250 g/f) caused a significant increase in the soybean yield (1180 kg/f) compared to the yield (1030 kg/f) resulting after treatment with a low dose of Dipel 2 \times (125 g/f). The addition of potassium carbonate (150 g/f) to either doses of Dipel (250 or 125 g/f) significantly increased the crop yield (1580 and 1450 kg/f) more than if Dipel 2 \times was used alone, but the difference in Dipel dose showed no effect when combined with potassium carbonate. The yield after treatment with Lannate (1270 kg/f) though significantly higher compared to the control, yet the effect was significantly higher compared to that of Dipel 2 \times alone.

Table 2. Yield assessment of soybeans as affected by a foliar spraying against *S. littoralis* at Beni-Suef governorate

Treatment	Dose/feddan (g)	¹ Mean yield (kg) per 100 m ² \pm S.E.	Feddan	Level of increase in yield
Control	–	14.50 \pm 3.041a	580	–
Dipel 2 \times	250	29.50 \pm 1.803bc	1180	2.03
	125	25.75 \pm 2.165b	1030	1.78
Dipel 2 \times + K ₂ CO ₃	250 + 150	38.00 \pm 1.871d	1580	2.62
	125 + 150	36.25 \pm 3.031d	1450	2.50
Lannate	300	31.75 \pm 5.309c	1270	2.19

¹ Means followed by the same letter are not significantly different at ($P = 0.05$) according to DUNCAN'S multiple range test.

3.2 Dust application

3.2.1 Effect on larvae

The data of dust application are presented in table 3. All treatments significantly reduced the population of larvae than the control. The effect was more pronounced after the addition of potassium carbonate to Dipel. The application of Dipel combined with potassium carbonate caused a higher significant reduction than if Dipel was dusted alone. Until the 7th day after dust application, Lannate treatment showed to be the best one, and reduction in larval population was significantly higher than for all other treatments. On the other hand, 10 days after application, the reduction in larval population after treatments with Lannate, Dipel 2 \times (250 g/f), Dipel 2 \times (250 or 150 g/f) combined with potassium carbonate were almost identical with insignificant differences and the percentages of larval reduction were 93.24, 91.97, 93.10, 92.11, respectively.

3.2.2 Effect on yield

Data on soybean yield as affected by dust application of Dipel and Lannate are reported in table 4. The factor of increase in the yield ranged between 1.34 and 1.91 for all treatments as compared to the control. For instance, the yield was almost identical in plots dusted with Dipel 2 \times (250 g/f) or Lannate (300 g/f). Lower doses of Dipel (125 g/f) caused a significant reduction in the yield, though this reduction was improved on using this Dipel dose combined with potassium carbonate. It is clear that Dipel 2 \times when used at the high dose (250 g/f) and combined with potassium carbonate led to the highest yield (1340 kg/f).

Table 4. Yield assessment of soybean as affected by dust application of Dipel and Lannate against *S. littoralis* larvae on soybean at Beni-Suef governorate

Treatment	Dose/feddan (g)	¹ Mean yield (kg) per		Level of increase in yield
		100 m ² ± S.E.	Feddan	
Control	—	17.50 ± 1.118a	700	—
Dipel 2×	250	29.75 ± 2.278c	1160	1.66
	125	23.50 ± 0.707b	940	1.34
Dipel 2× + K ₂ CO ₃	250 + 150	33.50 ± 3.082d	1340	1.91
	125 + 150	27.75 ± 1.785bc	1110	1.59
Lannate	300	28.00 ± 1.887c	1120	1.60

¹ Means followed by the same letter are not significantly different at (P = 0.05) according to DUNCAN's multiple range test.

4 Discussion

The foregoing results suggest that *B. thuringiensis* var. *kurstaki* HD-1 (Dipel 2×) may be recommended as an effective component of the future IPM programs against *S. littoralis* on soybean cultivations. When Dipel 2× at the rate of 250 g/f was sprayed combined with potassium carbonate (150 g/f), it gave the same larval reduction as that obtained after Lannate treatment (300 g/f). Also, the effectiveness of Dipel 2× (250 g/f) was as equal as that of a combination of Dipel 2× (125 g/f) and potassium carbonate. Assessment of the yield after spray application, however, indicates that the highest yield (1580 and 1450 kg/f) was obtained after treatment with Dipel 2× (250 or 125 g/f) combined with potassium carbonate, respectively. This was followed by Lannate, Dipel 2× (250 g/f) and Dipel 2× (125 g/f).

With dust application, some differences were observed. Thus, the highest larval reduction was attained after dusting with Lannate, Dipel 2× (250 or 125 g/f) combined with potassium carbonate and Dipel 2× alone (250 g/f). Dipel 2× at the rate of 125 g/f gave the least larval reduction. Evaluation of the yield revealed a high record (1340 kg/f) after dusting with a combination of Dipel 2× at the rate of 250 g/f and potassium carbonate (150 g/f). The yield with all other treatments (with the exception of Dipel 2× at the low rate 125 g/f) was almost the same and it ranged between 1110–1160 kg/f.

These results clearly show that one spray or dust application of Dipel 2× at the rate of 250 g/f combined with potassium carbonate (150 g/f) may substitute the carbamate chemical insecticide Lannate, to ensure the highest reduction in larval population of *S. littoralis* and to obtain the highest yield of soybeans. It appears that the addition of potassium carbonate increased the potency of Dipel 2×. This coincides with previous findings by SALAMA et al. (1985, 1986) relating this effect to the increase in the alkalinity of the insect midgut and thus solubilizing the crystal rendering it susceptible to the action of proteolytic enzymes (LECADET and MARTOURET 1965).

In this concern, it is worth to mention that the retarded effect of *B. thuringiensis* at sublethal doses (SALAMA et al. 1981) may be one of the factors that lead to the highest yield of the crop compared to other treatments which caused a comparatively more larval reduction 7–10 days after treatment.

Further large scale field experiments using this biological control agent will be published elsewhere.

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Zusammenfassung

Zum Einsatz von *Bacillus thuringiensis* Berliner und Lannate gegen *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) auf Sojabohnenpflanzen im Sprüh- und Stäubeverfahren

Die Wirksamkeit von *B. thuringiensis* var. *kurstaki* HD-1 (Dipel 2×), allein oder kombiniert mit Kaliumcarbonat, gegen *S. littoralis* wurde in Sojakulturen untersucht. Vergleichsmittel war das Carbamat Lannate. Die Präparate wurden im Sprüh- oder Stäubeverfahren ausgebracht. Die Ergebnisse zeigen, daß die Zugabe von Kaliumcarbonat die Wirksamkeit von Dipel verbesserte. Beide Applikationsverfahren sind für Bekämpfungseinsätze geeignet. Der kombinierte Einsatz von Dipel (250 g/f) und Kaliumcarbonat (150 g/f), der im Sprüh- bzw. Stäubeverfahren eine hohe Reduktion der Larvendichte (96,86 % bzw. 92,11 %) und einen 2,5- bzw. 1,6fachen Anstieg der Ernte erzielte, wird empfohlen. Die Ergebnisse zeigen, daß die Kombination von Dipel und Kaliumcarbonat in Zukunft eine wirksame Komponente in IPM-Programmen zur Kontrolle von *S. littoralis* in Sojakulturen darstellt.

References

- ANONYMOUS, 1982: Larvin Thiodicarb – A new selective second generation carbamate insecticide. Technical Information Union Carbide Agric. Products Co. Inc., R. T. P. North Carolina.
- ABDALLAH, M. D.; ABUL-NASR, S., 1970: Effect on *Bacillus thuringiensis* Berliner on reproductive potential of the cotton leafworm. Bull. ent. Soc. Egypt 4, 171–176.
- ABUL-NASR, S.; ABDALLAH, M. D. 1970: Lethal and sublethal action of *Bacillus thuringiensis* Berliner on the cotton leaf worm, *Spodoptera littoralis* (Boisd.), Bull. ent. Soc. Egypt 4, 151–160.
- ALMAZ, M. M.; EL-SAYED, M. M.; SHAHIN, A.; ABU-ZAHW, M. M.; EL-HERRAWI, M. A., 1986: Persistence of Thiodicarb residues on soybean plants under the normal field conditions. Bull. ent. Soc. Egypt 15, 187–190.
- DUNCAN, D. B., 1951: A significance test for differences between ranked treatments in an analysis of variance. Va. J. Sci. 2, 171–189.
- LECADET, M. M.; MARTOURET, D., 1965: The enzymatic hydrolysis of *Bacillus thuringiensis* Berliner crystals on the liberation of toxic fractions of bacterial origin by the cycle of *Pieris brassica*. J. Invertebr. Pathol. 7, 105–108.
- LEWIS, L. C.; LYNCH, R. E., 1978: Foliar application of *Nosema pyransta* for suppression of population of European corn borer. Entomophaga 23, 83–88.
- MORRIS, O. N., 1980: 1979 Canada-USA cooperative *Bacillus thuringiensis* spray trials against the spruce budworm, *Choristoneura fumiferana*. SIP XIII, Ann. Meeting, Univ. Washington.
- SALAMA, H. S.; FODA, M. S., 1982: A strain of *Bacillus thuringiensis* var. *entomocidus* with high potential activity on *Spodoptera littoralis*. J. Invertebr. Pathol. 39, 110–111.
- SALAMA, H. S.; FODA, M. S.; DULMAGE, H. T.; EL-SHARABY, A., 1983: Novel fermentation media for production of δ-endotoxin from *Bacillus thuringiensis*. J. Invertebr. Pathol. 41, 8–19.
- SALAMA, H. S.; FODA, M. S.; EL-SHARABY, A., 1981a: Potency of spore-δ-endotoxin complexes of *Bacillus thuringiensis* against some cotton pests. Z. ang. Ent. 91, 388–398.
- — — 1984a: Novel biochemical avenues for enhancing *Bacillus thuringiensis* endotoxin potency against *Spodoptera littoralis* (Lep.: Noctuidae). Entomophaga 29, 171–178.
- SALAMA, H. S.; FODA, M. S.; EL-SHARABY, A.; MATTER, M.; KHALAFALLAH, M., 1981b: Development of some Lepidopterous cotton pests as affected by exposure to sublethal levels of endotoxins of *Bacillus thuringiensis* for different periods. J. Invertebr. Pathol. 38, 220–229.
- — — 1985: Potential of some chemicals to increase the effectiveness of *Bacillus thuringiensis* Berliner against *Spodoptera littoralis* (Boisd.). Z. ang. Ent. 100, 425–433.
- SALAMA, H. S.; FODA, M. S.; SHARABY, A. 1986: Possible extension of the activity spectrum of *Bacillus thuringiensis* strains through chemical additives. J. Appl. Ent. 101, 304–313.
- SALAMA, H. S.; FODA, M. S.; ZAKI, F. N.; MOAWED, S. M., 1984b: Potency of combinations of *Bacillus thuringiensis* and chemical insecticides on *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. econ. Entomol. 77, 885–890.
- SALAMA, H. S.; MOAWED, S. M.; ZAKI, F. N., 1987: Effects of nuclear polyhedrosis virus – *Bacillus thuringiensis* combinations on *Spodoptera littoralis* (Boisd.) J. Appl. Ent. 104, 23–27.

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