EFFICACY AND PERSISTENCE OF A COMMERCIAL FORMULATION OF *BACILLUS THURINGIENSIS KURSTAKI* AGAINST LARVAE OF *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE) ON TWO HOST PLANTS

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INTRODUCTION

Using *Bacillus thuringiensis* (*B.t.*) as environmentally safe biocontrol agent for controlling polyphagous lepidopterous larvae showed different efficacy levels on a certain target pest in relation to the treated host plant. This result depends on the fact that certain plant species contain chemical components that affect the development of the microbe. Testing sap of different plants (*in vitro*) or feeding larvae on different host plants (*in vivo*) by many authors demonstrated this phenomenon, *e.g.,* Kushner and Harvey (1962), Smirnoff and Hutchison (1964),Kudler and Lesenko (1981) and El-Husseini *et al.*(2000). Merdan *et al.* (1975) and Atalla (1992) proved in both *in vitro* and *in vivo* tests that the Egyptian clover, *Trifolium alexandrinum* L. includes no such components. Accordingly, this crop was selected as an important economic host plant for controlling larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) with *B.t.* (El-Husseini, 1981 and Salama *et al.*, 1999). For the same reason, this crop was also selected to carry out the present study compared to the common castor bean leaves (*Ricinus cmmunis*) used mostly for rearing this pest and also for bioassying different pesticides.

MATERIAL AND METHODS

The commercial formulation Dipel DF based on *Bacillus thuringiensis* subsp. *kurstaki* was applied at the recommended rate of 200g/200L water/feddan on the Egyptian clover (*T.alexandrinum*) and on ricinus plants (*R.communis*) grown in the Experimental Station at the Faculty of Agriculture, Cairo University at Giza. A 20L-back motor sprayed was used for application of the biomaterial on both host plants on March 2^{nd}, 2007.

Samples of sprayed leaves were collected at zero-time (just after application), and then daily for the next 6 days. Sampled leaves were placed directly in the field into labeled half-liter plastic containers with perforated covers to avoid touching the sprayed material once again in the laboratory. At the same intervals, samples from untreated clover and ricinus leaves were similarly collected to serve as control. Containers with sampled plant material were transferred to the laboratory where larvae of *S.littoralis* were placed onto the treated and untreated host leaves by means of a fine brush. Twenty five newly hatched larvae (L1) were placed into each of four containers for each treated host plant. Other four containers from each treated host plant were provided each with 25 larvae of fourth instar (L4). The same procedure was applied each sampling time using untreated leaves from each host plant to serve as control. The larvae were left feeding on the sampled material for 24 hours. Thereafter, the treated leaves were replaced daily for one week by new untreated ones and larval mortality was recorded daily in both treatments and control. All tests were carried out under laboratory conditions of 25 ± 2 °C and 55-60% R.H.

RESULTS AND DISCISSION

1. Assaying versus *S.littoralis* L1

Assaying the sampled plant material sprayed with Dipel DF (*B.t.kurstaki*) versus *Spodoptera littoralis* larvae of L1, showed different mortality rates on both tested host plants (Table 1). The zero time sample resulted high mortality among larvae of *S.littoralis* on the 7^{th} day post feeding, being 92 and 90% for clover and ricinus, respectively. But the initial mortality 24 hours after treatment was higher on ricinus leaves (56%) than on clover (36%). Samples of the second day induced initial mortaliy of 25 and 48% on clover and ricinus, but respectively ended with 52 and 92% on the 7^{th} day post feeding. Samples collected three days after spraying *B.t.kurstaki* in the field, showed initial mortality after 24 hours clearly decreased to 15% on clover but remained by 48% for ricinus where both increased to 32 and 82%, respectively on the 7^{th} day post feeding. Such decrease in the efficacy of *B.t.* on clover is related to the fact that larvae of L1 tend to feed inside the folded apical leaves of the clover pants more than on the sprayed developed leaves, and thus they don't ingest enough *B.t.* material. On the other hand, ricinus leaves offered an ideal treated leaf surface showing higher mortality rates when compared with treated clover plants. Four days after application, the efficacy of *B.t.* decreased to 21% on sprayed clover on the 7th day compared to 58% on ricinus leaves. However, this result shows that the tested biomaterial lost 43.47, 62.21, 77.17, 90.21, 97.82 and 100% of its efficacy successively from the 2nd to the 7th day post application on clover due to the feeding behavior of the young *S.littoralis* larvae. Meanwhile, sprayed ricinus leaves showed high persistence of *B.t.* for the first three days after field application (Table 1), where efficacy decreased by only 8.88% on the 3rd day. Thereafter, it decreased to 35.55, 58.88, 73.33 and 95.55% on the 4th, 5th, 6th and 7th days , respectively. Previous studies by Attalah (1992) and El-Husseini *et al.* (2000) did not mention the feeding behavior of *S.littoralis* L1 on clover and its relation to ingested quantity of biomaterial sprayed on this crop in the field.

TABLE (I)

Efficacy and persistence of *B.t.kurstaki* (Dipel DF) versus L1 of *S.littoralis* on treated leaves of two host plants.*

Sampling day	Host plant	Accumulated mortality % among one week post feeding on the sample							Decreased efficacy %
		1	2	3	4	5	6	7	
Zero time	Clover	36	76	84	92	92	92	92	
	Ricinus	56	64	68	72	80	88	90	
2	Clover	25	28	52	52	52	52	52	43.47
	Ricinus	48	72	84	88	92	92	92	00
3	Clover	15	32	32	32	32	32	32	62.21
	Ricinus	48	58	60	68	78	80	82	8.88
4	Clover	3	21	21	21	21	21	21	77.17
	Ricinus	32	48	50	58	58	58	58	35.55
5	Clover	0	0	7	9	9	9	9	90.21
	Ricinus	30	31	31	31	36	37	37	58.88
6	Clover	0	1	2	2	2	2	2	97.82
	Ricinus	12	12	16	16	24	24	24	73.33
7	Clover	0	0	0	0	0	0	0	100
	Ricinus	2	3	3	4	4	4	4	95.55

* Mortality among control replicates remained by zero all the time.

2. Assaying versus *S.littoralis* L4

Sprayed plant leaves collected at the zero time induced 72% mortality among *S.littoralis* larvae of L4 fed on clover compared with 100% among those fed on ricinus five days post feeding on the treated host plant. One day after field application, the efficacy of *B.t.* remained stable on ricinus causing 100% mortality on the 5th day post feeding, while it deceased from 72 to 32% on clover plants at the same time being decreased by 55.55% than that of the previous day. The persistence of sprayed *B.t.* material under field conditions decreased clearly on the 3rd day after application; it caused 8 and 74% mortality in larvae of L4 on treated clover and ricinus on the 7th day post feeding, showing respective decrease in efficacy by rates of 88.88 and 26% on both host plants. A complete reduction (100%) of the *B.t.* efficacy was recorded on clover at the 4th day post field application, while it was decreased by 48, 89 and 100% on the 4th, 5th and 6th days on ricinus, respectively (Table 2).

TABLE (II)

Efficacy and persistence of *B.t.kurstaki* (Dipel DF) versus L4 of *S.littoralis* on treated leaves of two host plants.*

Sampling day	Host plant	Accumulated mortality % among one 6 days post feeding on the sample							Decreased efficacy %
		1	2	3	4	5	6	7	
Zero time	Clover	0	28	64	72	72	72	72	
	Ricinus	0	70	84	92	100			
2	Clover	0	4	12	28	32	32	32	55.55
	Ricinus	0	66	86	92	100			0
3	Clover	0	0	8	8	8	8	8	88.88
	Ricinus	0	22	46	52	66	74	74	26
4	Clover	0	0	0	0	0	0	0	100
	Ricinus	0	8	32	39	39	48	52	48
5	Clover	0	0	0	0	0	0	0	-
	Ricinus	0	0	0	4	9	11	11	89
6	Clover	0	0	0	0	0	0	0	-
	Ricinus	0	0	0	0	0	0	0	100

* Mortality among control replicates remained by zero all the time.

According to the *in vitro* and *in vivo* results of Merdan *et al.*(1975) and Atalla (1992), higher mortality rates among *S.littoralis* larvae are to be expected in case of clover as a host plant free from antibacterial components. Thus, the present low rates of larval mortality on this host plant may seem unusual. But the fact that fourth larval instar (L4) of *S.littoralis* feed by cutting and chewing the tested plant leaves; and thus they differ in their feeding behavior from those of the first instar (L1) that mostly feed on the lower mesophyl layer of their host plant leaves could explain the present results. Accordingly, such feeding behavior affects the ingested amount of the sprayed biomaterial on leaves of its host plants.

Also, the nature of developing foliage in clover being with many folded young leaves on top of the plant that received the sprayed biomaterial only on the external surface of such leaves leaving the inside surfaces almost free from the applied material has an important value when bioassying sprayed entomopathogens such as *B.t.* formulations versus lepidopteran larvae. Thus, it is supposed that the ingested amount of *B.t.* could be lower in case of assaying treated clover versus L1 of *S.littoralis* larvae than those of L4. Results presented in Tables 1 and 2 could explain this important relationship. The relationship between feeding behavior of young lepidopterous larvae and ingested dose of sprayed *B.t.* was early studied by El-Husseini and Sermann (1977) in the tortricid *Pandemis heparana* Den.& Sciff., and El-Husseini and Afifi (1981) in *Earias insulana* L.

SUMMARY

Efficacy and persistence of the *Bacillus thuringiensis kurstaki* formulation Dipel DF (200g/feddan) was tested against larvae of both L1 and L4 of the cotton leafworm, *Spodoptera littoralis* (Boisd.) on the Egyptian clover (*Trifolium alexandrinum*) and castor bean (*Resinus communis*) leaves under field and laboratory conditions. Results showed that persistence of *B.t.* under field conditions lasted 3 days. Meanwhile, the efficacy against larvae was higher on ricinus than on clover due to both the feeding behavior of the L1 and L4 larvae on both crops, and the nature of plant leaves growth that defines the leaf surface receiving the sprayed biomaterial.

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