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Shelf-life of spicturin® (*Bacillus thuringiensis Berl. var. gallariae)* during storage and its efficacy against *Plutella xylostella* (L.) on cauliflower

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Abstract : The shelf-life studies were conducted to determine the shelf-life of SPICTURIN® (*Bacillus thuringiensis* Berl. var. *gallariae*) against *Plutella xylostella* L. on cauliflower by two methods *viz.*, from the unopened samples and opened sample of Spicturin. In the first method, 18 fresh samples of Spicturin were stored at room temperature (25 to 33°C) and each month one sample was opened for bioassay and enumeration of spore load. The bioassays were conducted for 18 months continuously by leaf-dip method. In the second method, first month opened sample was stored at room temperature and used for similar study. The present results indicated that Spicturin samples were found to maintain the spore load of 10^7 spores ml^{-1} for 18 months and there was not much reduction in viable spores in the first month opened sample. However, there were differences in the initial spore load itself in the first method. From the 18 months storage study on Spicturin, the results of both the methods exhibited that the mortality of the second and third instar larvae of *P. xylostella* varied from 95.00 to 85.00 and 85 to 75.00 per cent respectively for Spicturin 4ml L^{-1} which was higher when compared to lower doses of 3 and 2ml L^{-1}; however, it was on par with them. (***Key words*** : *Bacillus thuringiensis* Berl. var. *gallariae*, Storage, *Plutella xylostella* L, Cauliflower)

Bacillus thuringiensis Berliner (*B.t*) is one of the important biocontrol agents for the management of lepidopteran pests. The commercial formulations of *B.t.* should have the desirable qualities for effective storage and control of the pests. The product must be an excellent formulation with good shelf-life (Watkinson, 1991). The pathogen must remain viable during storage and in the environment long enough for better contact to the pest, the formulation should have been properly standardized with good shelf-life during storage (Fuxa, 1996). Moore and Prior (1996) reported that most of the mycoinsecticides having a short shelf-life even with cold storage, required an estimated range from three to 18 months or even longer period for effective storage. Bryant (1991a) reported that the stability of potency of liquid *B.t.* formulations was lower than that of dry formulations and requires temperature control for long term storage. Formulation of product affect the success of microbial insecticide approach to manage the pests. The new *B.t.* formulation, Spicturin was developed from *B.t.* var. *galleriae* which is specific to lepidopteron pests. The present study was taken to evaluate the shelflife of the new product against diamond back moth, *Plutella xylostella* (L.), a major pest of cauliflower.

Materials and Methods

The shelf-life studies were conducted to determine the shelf-life of Spicturin by two methods *viz.*, from the unopened samples and opened sample of Spicturin.

Shelf life of unopened Spicturin sample opened in different months

In this method, 18 fresh samples of Spicturin were stored at room temperature (25 to 33°C) and each month one sample was opened for bioassay and enumeration of spore load. The bioassays were conducted for 18 months continuously by leaf-dip method. The bioassays were conducted to evaluate the toxicity of Spicturin against second and third instar larvae of *P. xylostella* on its host plant, cauliflower.

Enumeration of B.t. spores in Spicturin formulation

Enumeration of the viable *B.t.* spores in Spicturin formulation was performed using spread plate technique (David, 1970). The formulation sample was serially diluted (10 fold dilution) and 100 ml of at least three dilutions (10^{-6}, 10^{-7} and 10^{-8}) were spread on nutrient agar (Lapage *et al.* 1970) plates in triplicate. Inoculated plates were incubated at 30°C. Colony count was made after 24h. Representative colonies were examined after 24 to 36h under phase-contrast microscope for crystal forming phenotype.

Leaf dip method

The following five treatments were used against second and third instar larvae of *P. xylostella* in the bioassay studies as per the method followed by Justin, (1987).

Treatments

1. Spicturin - 2ml L^{-1}
2. Spicturin - 3ml L^{-1}
3. Spicturin - 4ml L^{-1}
4. Chlorpyriphos (Standard insecticide) - 2ml L^{-1}
5. Control

Replications : Four @ 10 larvae/replication.

One hundred ml of aliquot for each concentration was prepared in 250ml conical flask. Teepol was added at the rate of 1ml per litre of water (0.001%) as a surfactant. The leaves of respective hosts were used for each treatment. The leaves were immersed in the suspension for 30 sec., the excess fluid was removed by uniform jerking and later dried in shade. The treated leaves with different concentrations were placed inside the plastic containers separately. A moistened filter paper (No.41) was provided below the leaf to maintain the turgidity of leaves. In each container, ten larvae of each instar with uniform size pre-starved for 12h were released and was covered with a muslin cloth and secured by a rubber band. The larvae were allowed to feed on the treated leaf for 24 after which they were transferred to fresh leaves of respective host plant. The treatments were replicated four times. Observations were made at 12h interval for the larval mortality and continued till the surviving insects pupated.

Shelf life of Spicturin sample opened in first months

In this method, the samples opened in the first month were stored at room temperature (25 to 33°C) and tested every month continuously upto 18 months as in the previous method.

Results and Discussion

Shelf-life of unopened Spicturin samples opened in different months :

Viable B.t. spores in Spicturin samples opened in different months

The results of the eighteen months study in the first method indicated that there was not much reduction in the viable spores (10^7 spores/ml) which ranged from 81 ± 4.5 to $42 \pm 3.4 \times 10^7$ spores/ml from first to eighteenth sample.

Toxicity of Spicturin to P.xylostella

The results of the storage study on spicturin samples opened in different months indicated that the mortality of the second instar larvae of *P.xylostella* varied from 85.00 to 65.00 and from 95.00 to 80.00 per cent in Spicturin @ 3 and 4ml L^{-1} respectively (Table 1). The mortality of third instar larvae varied from 72.50 to 60.00 and from 85.00 to 75.00 per cent in Spicturin @ 3 and 4ml L^{-1} respectively.

The standard insecticide, chlorpyriphos @ 2ml L^{-1} recorded cent per cent mortality of second and third instar larvae of *P. xylostella* during first month study and it was significantly superior to Spicturin @ 4 and 3ml L^{-1} which were on par with each other. Spicturin @ 2ml L^{-1} recorded lower mortality of second and third instar larvae of *P. xylostella* which varied from 67.50 to 50.00 and from 57.50 to 47.50 per cent respectively, however, it was on par with Spicturin @ 3ml L^{-1}.

Shelf-life of Spicturin samples opened in first month : Viable spores in Spicturin formulation

The results of the 18 months study for the first month also indicated that there was not much reduction in the viable spores (10^7 spores/ml) which ranged from 45+4.6 to 42+3.4x10^7 spores per ml over a period of 18 months.

Toxicity of first month opened Spicturin sample to P. xylostella

The results of the 18 months bioassay for month opened sample indicated that the mortality of the second instar larvae of *P. xylostella* varied fro 82.50 to 70.00 and from 92.50 to 80.00 per cent in Spicturin @ 3 and 4ml.l respectively. The mortality of the third instar larvae varied from 75.00 to 65.00 and from 85.00 to 75.00 per cent in Spicturin @ and 4ml L^{-1} respectively.

The results of the bioassay study in the first month indicated that Chlorpyriphos @ 2ml L^{-1} recorded cent per cent mortality of second and third instar larvae of *P. xylostella* which was significantly superior to Spicturin @ 4 and 3ml L^{-1} which were on par with each other. The larval mortality of second (67.50 to 47.50) and third (60.00 to 47.50) instar larvae of *P. xylostella* were lower in Spicturin @ 2ml L^{-1} however it was on par with Spicturin @ 3ml L^{-1}. Similar trend was observed in the remaining months.

Shelf-life of Spicturin formulation

The shelf-life of *B.t.* formulation was estimated for effective storage as suggested by Moore and Prior (1996). The shelf-life of Spicturin was evaluated by enumerating the spore load and also its efficacy against the second and third instar larvae of *P. xylostella* on cauliflower over a period of 18 months in two methods *viz.* unopened samples opened in different months and samples opened in the first month.

In the present study, the viable spores of Spicturin formulation were enumerated using Nutrient Agar (NA) plates (Lapage *et al.* 1970). Since all the colonies developed in the NA plates and known to form positive crystals, all the colonies were identified as *Bacillus thuringiensis.* The present results indicated that Spicturin samples were found to maintain the spore load of 10^7 spores ml^{-1} and there was much reduction in viable spores in the first month opened sample. However, there were differences in the initial spore load itself in the first method. The viable spores load of Spicturin ranged from 81+4.5 to 42+3.4x10^7 spores ml^{-1} from the samples opened in first 18 months and from 44+4.6 to 42+3.4x10^7 spores/ml in the Spicturin sample opened in the first month for a period of 18 months.

Table 1. Toxicity of spicturin opened in different months against second instar larvae of *P.xylostella*

Treatment	Mortality (%)																	
	1 May 98	2 June	3 July	4 Aug.	5 Sep.	6 Oct.	7 Nov.	8 Dec.	9 Jan.99	10 Feb.	11 Mar.	12 Apr.	13 May.	14 June	15 July	16 Aug.	17 Sep.	18 Oct.
Spicturin 2 ml L^{-1}	65.00 (53.34)c	67.50 (55.23)c	65.00 (53.34)c	65.00 (53.33)c	62.50 (52.24)c	60.00 (50.78)c	60.00 (50.77)c	62.50 (52.23)c	60.00 (50.78)c	60.00 (50.78)c	60.00 (50.78)c	57.50 (49.32)c	55.00 (47.87)c	55.00 (47.87)c	55.00 (47.88)d	50.00 (44.99)d	50.00 (44.66)d	50.00 (44.99)d
Spicturin 3 ml L^{-1}	82.50 (65.28)bc	85.00 (67.21)bc	80.00 (63.43)bc	80.00 (63.43)bc	80.00 (63.44)bc	75.00 (60.00)bc	77.50 (61.69)bc	75.00 (60.00)bc	75.00 (60.00)bc	75.00 (60.00)bc	75.00 (60.00)bc	75.00 (60.00)bc	75.00 (60.00)bc	67.50 (55.23)bc	65.00 (53.77)c	65.00 (53.77)c	65.00 (53.77)c	65.00 (53.77)c
Spicturin 4 ml L^{-1}	92.50 (74.13)b	92.50 (74.14)b	95.00 (77.08)b	92.50 (74.12)b	92.50 (74.12)b	90.00 (71.57)b	90.00 (71.58)b	90.00 (71.57)b	87.50 (69.31)b	85.00 (67.22)b	85.00 (67.22)b	85.00 (67.23)b	82.50 (65.28)b	82.50 (65.28)b	82.50 (65.28)b	80.00 (63.80)b	80.00 (63.80)b	80.00 (63.80)b
Chlorpyri -phos 2ml L^{-1}	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a	100.00 (90.00)a
Control	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d	0.00 (0.00)d

Figures in the parentheses are arcsine transformed values
In a column, means followed by a common letter are not significantly different by DMRT (P=0.05)

In the present investigation on the toxicity of Spicturin samples, the results showed the mortality of second and third instar larvae of *P. xylostella* was higher at higher doses of Spicturin @ 4ml L^{-1}, however, it was on par with other doses of 3 and 2ml L^{-1}. This results is in consonance with the findings of Justin (1987) who reported that early instars were highly susceptible when compared to late instars of *P. xylostella* to *B.t.* formulations, Bactospeine and Delfin.

From the 18 months storage study on Spicturin, the results of both the methods exhibited that the mortality of the second and third instar larvae of *P. xylostella* varied from 95.00 to 85.00 and 85 to 75.00 per cent respectively for Spicturin 4ml L^{-1}. From the above results, it could be inferred that the reduction in per cent mortality of larvae was to the extent of 15.00 per cent from the first to 18th month for *P. xylostella*. The reduction in effectiveness of Spicturin might be due to considerable reduction in spore viability or the denaturation of the spore crystal complex. Earlier, Angus (1967) also reported that the parasporal crystal was responsible for the toxicity of *B.t.* and its toxicity varied with crystal count but was independent of the number of spores present in the formulation. The variations observed in the mortality of larvae of different instars were dependent upon the midgut pH of the test insect, developmental stage of the insect, food source, the rate of food consumption, food particle size, *etc.* as reported by Martin (1994). Bryant (1991b) reported that the storage of *B.t.* products at elevated temperature (32°C) would gradually denature the product and hence temperature control was suggested for longterm storage. He also observed that the stability of liquid *B.t.* formulation was lower than that of dry formulations.

The use of conventional *B.t.* based products is often contained by short shelf life, low potency, lack of systemic activity and the presence of less viable spores (Lambert and Peferoen, 1992). But the new product have good shelf life in storage, maintaining the viability of the spores and the shelf life of the formulation appeared to be desirable to cover two cropping seasons within a year as reported by Moore and Prior (1996). The present investigation on the indigenous *B.t.* formulation, Spicturin can be stored for 18 months at room temperature for the management of *P. xylostella* on cauliflower in two cropping seasons.

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