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Computation of LC_{50} against *Spodoptera litura* (Fab.) for *Bacillus thuringiensis* isolates from native soil samples of Andhra Pradesh

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Abstract
A total of 203 *Bacillus thuringiensis* isolates were collected from soil samples native to southern zone of Andhra Pradesh distributing in Chittoor, Kadapa and Nellore districts. These isolates were tested for their efficacy against third instar *Spodoptera litura* in laboratory bioassays with leaf dip method. Out of 203 isolates 21 isolates (C44, C33, C59, C63, C79, C92, C97, C105, C134, C212, K18, N3, N30, N44, N48, N58, N115, F287, F468, F493 and F504) were observed with a mortality of more than 75 per cent against third instar *S. litura* larvae. These isolates were further tested for fixing the lethal doses to kill 50 per cent third instar *S. litura* larval population. Among the 21 isolates, the LC_{50} values were in the range of 9.59×10^4 to 1.88×10^6 and in HD-1 lowest LC_{50} of 9.56×10^4 followed by 9.76×10^4 in F493 and N30 with 1.90×10^5.Whereas, highest lethal concentration to kill 50 per cent *S. litura* was 1.88×10^6 spores ml^{-1} in C212. Lowest time to kill 50 per cent was noticed in HD-1 (61.99 h), followed by F493 (78.52 h) and F468 (74.28h) and N115 (88.68h). Among the 21 isolates, F493, N30, F468, N115 can be used to develop *Bt* based biopesticide formulations for *S.litura* control.

Keywords: *Spodoptera litura*, *Bt* isolates, native soils, LC_{50}

Introduction
Bacillus thuringiensis (*Bt*) is a Gram-positive bacterium, stands out representing approximately 95% of microorganisms used in biological control of agricultural pests in different cultures, which accounts for 1.3 per cent of total pesticides (Ramanujam *et al*., 2014) [11]. Besides the economic aspect and the safety to human health, this bacterium is the most promising for the production of bio-pesticides and plant resistant to insects, associated with environmental preservation. The research worldwide revealed that, thousands of isolates were collected from various sources and tested for their efficacy against different insect pests. But very few isolates were found specific to lepidopteran pests such as *Spodoptera litura* which is a polyphagous pest with multiple generations in a year. For controlling of this pest, farmers are applying at least 4-5 insecticidal sprays in groundnut during post rainy season particularly in January to March and sometimes combination pesticides are also being used. Hence, identifying a bio-product based on active ingredients of *Bt* is highly useful to avoid problems like environmental pollution, pesticide residues, development of resistance to insecticides, resurgence of secondary pests *etc*., In this context, the present studies were conducted at Department of Entomology, Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati for identifying *Bt* isolates with higher efficacy in controlling *S.litura*.

Materials and Methods
Isolation of *Bt* from soil samples
A total of 925 soil samples were collected from different areas covering cultivated soils, fallow lands, forest soils of Chittoor, Kadapa and Nellore districts. The samples were collected at a depth of 10-15cm in sterile polythene bags by using sterilized spatula and brought to laboratory for further processing. These soil samples were stored at 4°C until processed for isolation of *Bt.* These samples were processed for isolating *Bt* using Sodium acetate selection method suggested by Travers *et al.,* (1987) [15] with slight modifications.

Bioassay of Native *Bt* isolates against *S. litura*
Native *Bt* isolates were used for bioassay study against *S. litura* along with reference strain *Bt sub sp. kurstaki* (HD1) to ascertain their insecticidal activity. Individual isolate was streaked on plain Luria Bertani agar plates and incubated overnight at 37°C. One loop of overnight cultures was inoculated in Luria broth and kept for sporulation under shaking condition at

28°C for 24h.

Bioassay was followed by leaf dip bioassay method developed by Shelton *et al.* (1993) [14] was adopted. Groundnut compound leaf containing four leaflets was dipped for 10 minutes into *Bt* culture broth (5×10^8 CFU/ mL) containing 0.2 per cent Triton X-100, then kept leaf for air drying till leaf surface free from moisture. After drying, the petiole of leaf was swabbed with wet cotton to maintain leaf succulence and turgidity. Two compound leaves were used for one replication, which was placed in a Petri plate. Ten larvae were released per one replication. HD-1 served as a reference strain. The leaf dipped in distilled water served as control. The larval mortality was assessed after 72h at regular intervals.

Determination of lethal concentration (LC_{50})

Forty mL L.B. broth was taken in conical flask, sterilized medium at 121°C for 15 minutes. The isolates *viz*., C33, C44, C59, C63, C79, C97, C105, C134, C212, K18, N3, N30, N44, N48, N58, N93, N115, F287, F468, F493, F504 along with reference strain (HD1) were further used for determining LC_{50} values. After cooling inoculated with one loop of each *Bt* isolate into conical flask containing L.B. broth. Then L.B. broth kept in shaker at 300 rpm for 3 days. Serial dilutions were prepared at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. 100 µl of each dilution was taken and spread on Luria agar plate with 'L' rod. The plates were kept in incubator for overnight at 37°C. After 24h colony count was taken. Based on the CFUs the concentrations for bioassay was prepared and finally made the volume to 30mL for adopting leaf dip.

Groundnut compound leaf containing four leaflets were dipped into culture broth containing 0.2 per cent Triton X-100 for 10 minutes. Then the leaves were kept for drying. After drying leaf petiole was swabbed with wet cotton to maintain leaf turgidity and dip the leaf in *Bt* strains with different dilutions. One Groundnut leaf for one replication was placed in a petriplate. Ten second instar larvae were released per one replication. HD-1 served as a reference strain. The leaf dipped in distilled water served as control. The larval mortality was assessed after 72 h and LC_{50} values were determined using probit analysis.

Results and Discussion

The toxicity of *Bt* is a dose dependent phenomenon and the dose acquired by an individual is directly related to the quantity of treated food consumed. In present studies, 21 isolates isolated from soil samples of Chittoor, Kadapa, Nellore and Forest ecosystem *viz.*, C44, C33, C59, C63, C79, C92, C97, C105, C134, C212, K18, N3, N30, N44, N48, N58, N115, F287, F468, F493 and F504 were observed with a mortality of more than 75 per cent against third instar *S. litura* larvae (Table.1). These isolates were further tested for fixing the lethal doses to kill 50 per cent third instar *S. litura* larval population.

Among the 21 isolates, the LC_{50} values were in the range of 9.59×10^4 to 1.88×10^6 and in HD-1 lowest LC_{50} of 9.56×10^4 followed by 9.76×10^4 in F493 and N30 with 1.90×10^5.Whereas, highest lethal concentration to kill 50 per cent *S. litura* was 1.88×10^6spores ml^{-1} in C212. Lowest time to kill 50 per cent was noticed in HD-1 (61.99 h), followed by F493 (78.52 h) and F468 (74.28h) and N115 (88.68h) (Table.2 & Fig. 1).

The results of present studies are in same way with the findings of Sharma (2000) [13], who reported a mortality of 66.66 to 100 per cent with five *Bt* formulations against *S. litura* and *Spilarctia oblique* under controlled conditions at 26±1°C and 75 per cent relative humidity which were on par with endosulfan. Similarly, Hassan *et al*. (2011) [4] reported that four Syrian isolates (SSy125-c, SSy141-c, SSy111-c and SSy112-c) identified as *Bt kurstaki* showed higher toxicity than the standard strains (HD-1 and HD73) which are the active ingredients of commercial preparations commonly used as bio-insecticides. The studies of Lalitha *et al.* (2012) [5] revealed a mortality of 16.67 to 94.44 per cent with *Bt* isolates against second instar larvae of *H. armigera* where *Bt* isolates 122 and 22 recorded 83.33 per cent mortality and was statistically on par with HD-1. At the same time Al-Otaibi (2013) reported a higher positive efficiency *Bt* isolates in spore+ crystal mixtures @ 10^9 CFU ml^{-1} at 168h after treatment against second instar *S.littoralis* larvae and Ricardo *et al.* (2000) also reported a mortality of 100 and 80.4 per cent in second instar larvae of *S.frugiperda* with the suspensions of *Bt aizawai* HD 68 and *Bt thuringiensis* 4412, containing 3×10^8 cells ml^{-1}.

In addition to this, Azzouz *et al*. (2014) [2] also reported a higher toxicity of two *Bt* strains-endotoxin production by two *Bt* isolates that had high insecticidal activity against *S. littoralis*. Added to these, Rabari *et al*. (2016) [10] reported that, treatments with neem oil @ 0.5 per cent (53.87%) and *Bt* 5×10^7 spores mg^{-1} @ 0.2 per cent (50.50%) found to be as effective as thiodicarb in *S. litura* larval mortality. Added to these findings, Pooja *et al*. (2013) [8] reported a cumulative mortality of 83.33 per cent with the *Bt* isolate DBT153 against *Plutella xylostella*collected from hill ecosystems in Coorg district.

Similar to results of present study, Devaki and Krishnayya (2004) reported LC_{50} values of 2.42×10^4, 2.80×10^4, 3.55×10^4 spores ml^{-1} in three commercial *Bt* formulations *viz*., Dipel8L, Delfin WG and Halt WP against third instar *S. litura* larvae.

The present studies were comparable with Pandey *et al*. (2009) [7] who reported that *Bt aizawai* HD-137 was more toxic with LC_{50} of 0.50ppm than *Bt kurstaki* HD-1 (LC_{50} 1.12 ppm) to the 1st instar larvae of *S. litura*. *Bt aizawai* HD-137 produces *cry*1C toxin (Porcar *et al*., 2000) [9] which is highly toxic to the larvae of *S. litura*. Further, Young *et al*., (2007) [16] reported the LC_{50} of the crystal proteins produced by isolate 2385-1 was two-fold lower (0.78×10^5CFU ml^{-1}) to *P. xylostella* compared to *Bt* subsp. *Kenyae* (1.61×10^5 CFU ml^{-1}). Similarly 2385-1(8.1×10^5 CFU ml^{-1}) showed a high toxicity towards *S. exigua* larvae, whereas *Bt.*subsp. *kenyae* was absolutely nontoxic to *S. exigua* larvae($>100\times10^5$ CFU ml^{-1}). Higher efficacy might be due to the kind of midgut proteases and their role in protoxin activation and the presence of midgut receptor for *Bt* toxins and their binding affinity are some of the host mediated factors that determines specificity and toxicity of a *Bt* strain/toxin towards a given insect species. As against this, Mohan *et al*. (2014) [6] reported a low toxicity of *Bt* HD-1strain to *S. litura* and also HD-73 was non-toxic to *S. litura* and *S. oblique*.

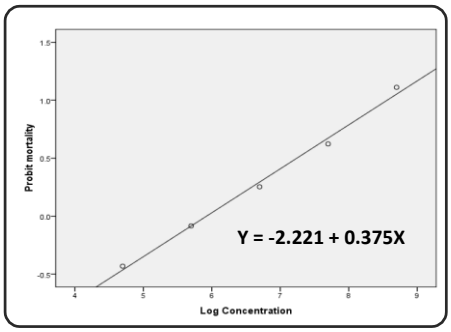
Table 1: Efficacy of native *Bt* isolates against third instar *S.litura* under laboratory bioassay at 5×10^8 CFU/mL

S. No.	Isolate	Larval Mortality (%)	Place of collection	Crop/ location
1	C33	80.00	Pudi	Mango
2	C44	76.67	Thondamanadu	Cultivated Fallow
3	C59	76.67	Paipalem	Tagetus
4	C63	76.67	Chinn Ubba	Ragi
5	C79	83.33	Vanamasinapalle	Potato
6	C97	83.33	Adavibuduguru	Fodder
7	C105	76.67	Musalipedu	Cultivated Fallow
8	C134	83.33	M.Kothuru	Cultivated Fallow
9	C212	83.33	Vengalathur	Sugarcane
10	K18	86.67	H.Kothapalli	Fallow
11	N3	83.33	Mambattu	Groundnut
12	N30	86.67	Chembedupalem	Grass
13	N44	76.67	Venkatagiri	Jack fruit
14	N48	76.67	Petlur	Neem
15	N58	76.67	Venkatagiri	Cultivated Fallow
16	N93	76.67	Chaaganam	Hill
17	N115	76.67	Kasumur	Eucalyptus
18	F287	76.67	Talakona	Forest down
19	F468	86.67	Bhakarapet ghat	Forest dowm
20	F493	100.00	Talakona	Forest up
21	F504	76.67	S.V. Zoo park	Forest area
22	HD-1	96.66	Standard	-

Table 2: Dose fixation of native *B. thuringiensis* isolates against third instar *S. litura* larvae

S. No.	*Bt* isolates	Regression equation	LC_{50} values CFU mL^{-1}	Fiducial limits CFU mL^{-1}		LC_{90} values CFU mL^{-1}	Fiducial limits CFU mL^{-1}		LT_{50} (h)	LT_{90} (h)
				Lower	Upper		Lower	Upper		
1	C33	Y = -2.221+0.375X	8.39×10^5	$1.20x\ 10^5$	3.01×10^6	2.20×10^9	2.40×10^8	3.20×10^{11}	131.32	180.43
2	C44	Y =-2.751+0.457X	1.04×10^6	2.51×10^5	3.15×10^6	6.68×10^8	1.20×10^8	1.85×10^{10}	137.56	183.89
3	C59	Y =-2.278+0.390X	7.00×10^5	1.05×10^5	2.51×10^6	1.36×10^9	1.74×10^8	1.19×10^{11}	113.37	206.57
4	C63	Y =-2.716+0.402X	5.67×10^6	1.53×10^6	2.15×10^7	8.70×10^9	8.49×10^8	1.29×10^{12}	109.89	197.44
5	C79	Y =-1.572+0.287X	3.02×10^5	7.36×10^3	1.72×10^6	8.88×10^9	4.20×10^8	9.32×10^{13}	97.83	174.34
6	C97	Y= -2.410+0.441X	2.94×10^5	4.26×10^4	9.91×10^5	2.39×10^8	4.60×10^7	6.39×10^9	97.83	174.34
7	C105	Y =-0.164+0.357X	1.15×10^6	1.67×10^5	4.49×10^6	6.80×10^8	9.87×10^7	4.47×10^{10}	125.97	202.22
8	C134	Y= -2.208+ 0.395X	3.90×10^5	4.72×10^4	1.43×10^6	6.84×10^8	9.87×10^7	4.45×10^9	109.83	174.48
9	C212	Y =-2.771+ 0.525X	1.88×10^6	3.34×10^4	5.61×10^5	5.15×10^7	1.39×10^8	5.57×10^8	101.33	166.99
10	K18	Y =-2.133+0.399X	2.20×10^5	2.04×10^4	8.59×10^5	3.58×10^8	5.82×10^7	1.71×10^{10}	128.43	224.42
11	N3	Y =-2.267 +0.425X	2.18×10^5	2.45×10^4	7.89×10^5	2.27×10^8	4.19×10^7	7.25×10^9	129.25	175.33
12	N30	Y =-2.616+0.496X	1.90×10^5	2.97×10^4	6.01×10^5	7.33×10^7	1.82×10^7	9.72×10^8	95.70	180.74
13	N44	Y =-1.891 +0.315X	9.93×10^5	9.16×10^4	4.59×10^6	1.15×10^7	6.42×10^7	2.59×10^{13}	116.47	193.62
14	N48	Y =-2.256 +0.365X	1.50×10^6	2.55×10^5	5.75×10^6	4.85×10^9	4.39×10^8	1.22×10^{12}	107.79	233.62
15	N58	Y =-2.687 +0.453X	8.42×10^5	1.86×10^5	2.57×10^6	5.65×10^8	1.02×10^8	1.59×10^{10}	92.78	206.34
16	N93	Y =-2.398 +0.387X	1.59×10^6	3.09×10^5	5.68×10^6	3.27×10^9	3.56×10^8	4.22×10^{11}	115.31	196.39
17	N115	Y =-2.689+0.434X	1.57×10^6	3.74×10^5	4.94×10^6	1.40×10^9	2.13×10^8	6.15×10^{10}	88.68	205.28
18	F287	Y =-2.709+0.435X	1.71×10^6	4.22×10^5	5.40×10^6	1.52×10^9	2.26×10^8	7.05×10^8	121.37	214.56
19	F468	Y =-3.490+0.605X	5.93×10^5	1.84×10^5	1.46×10^6	7.82×10^7	2.38×10^7	5.75×10^8	74.28	202.63
20	F493	Y=-2.647+0.531 X	9.76×10^4	1.26×10^4	3.17×10^5	2.54×10^7	7.17×10^6	2.58×10^8	78.52	114.07
21	F504	Y=-2.112+0.3219X	2.62×10^6	4.22×10^5	1.20×10^7	2.03×10^{10}	1.09×10^9	3.54×10^{13}	89.65	248.49
22	HD-1	Y =-2.252+0.452X	9.59×10^4	8.13×10^3	3.67×10^5	6.56×10^7	1.48×10^7	1.21×10^9	61.99	121.64

*Y=a+bx; where Y=probit;X=Concentration (CFU/mL); a=intercept; b=slope



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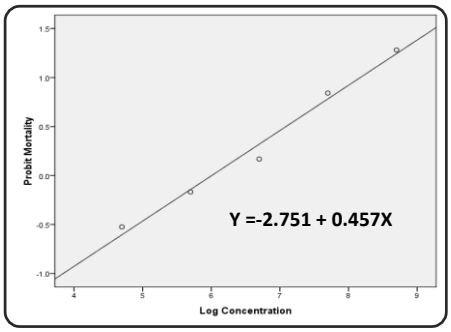

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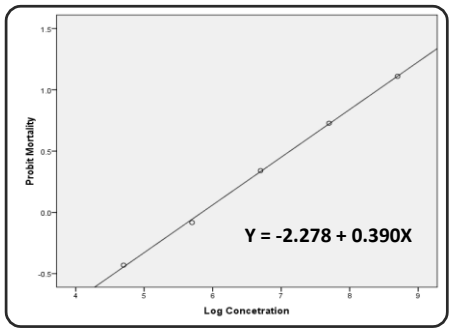

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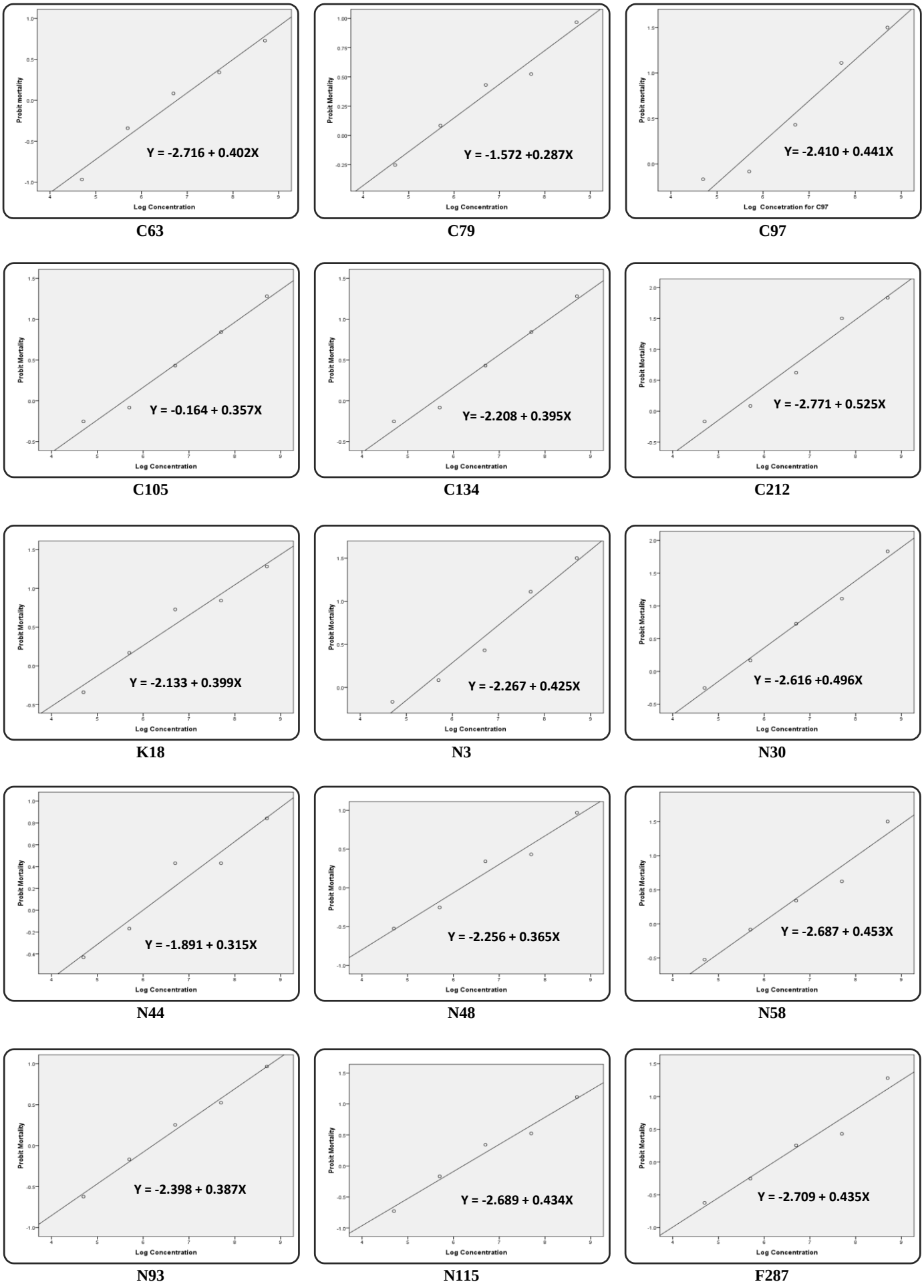
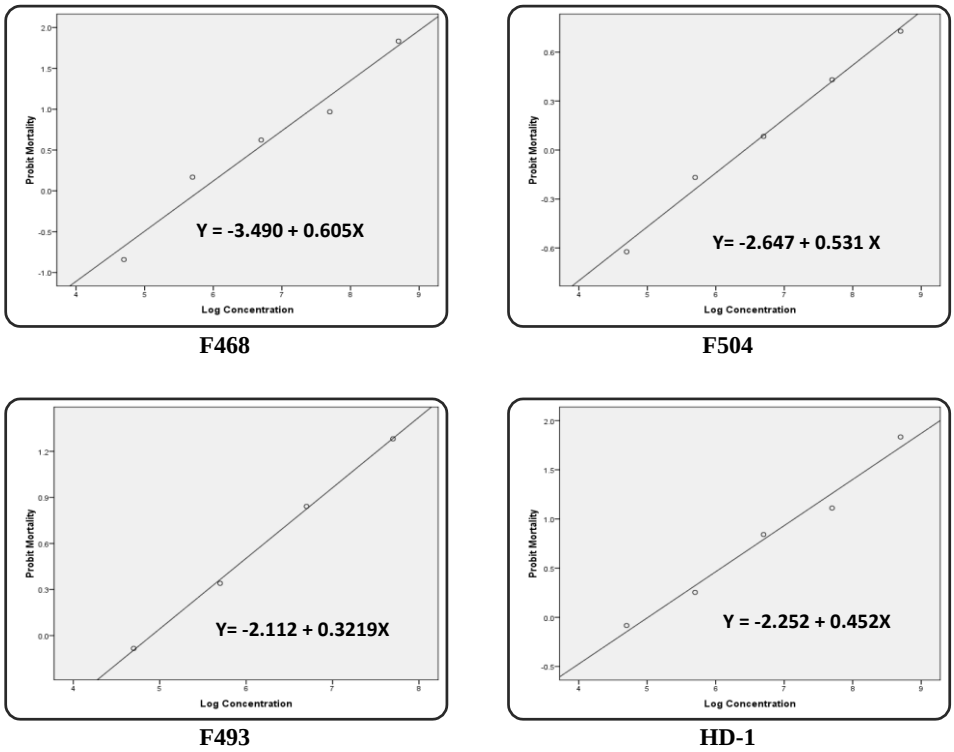


Fig 1: Log concentration-probit mortality regression lines for *B. thuringiensis* isolates against third instar *S. litura* larvae



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