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In vitro compatibility and efficacy studies of entomopathogenic fungi *Beauveria bassiana* with commonly used biorational and chemical pesticides against *Spodoptera litura* (Fabricius)

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Abstract
In vitro compatibility of selected entomopathogenic fungi with botanicals and chemical insecticides at field recommended concentrations indoxacarb, Spinosad, Neem oil and NSKE were non-toxic to the test strains of *B. bassiana* (Bb-L-2) did not show significant reduction in radial growth. The insecticide Diclorovos (DDVP) recorded 100 per cent reduction in radial growth of test strains at field recommended concentration. The joint action of microbial agents (bacteria, viruses and fungi) revealed that the combination of pathogens did not prove superior to individual effect. All the combination of entomopathogenic fungi (*B. bassiana* (Bb-L-2) strain with microbial agents were within the critical limits of additive effect and combination with insecticides viz., Spinosad 45EC @0.009%, neem oil 5% and NSKE 5%, which produced the synergism reaction.

Keywords: *Beauveria bassiana*, spinosad, indoxacarb, novaluron, cartap hydrochloride, tebuconazole, azoxystrobin, chlorothalonil, propiconazole, *Spodoptera litura*

Introduction
During the last 30 years, there has been growing interest in the use of entomopathogenic fungi as control agents of insect pests as part of general movement towards IPM and away from reliance on chemical pesticides. Worldwide, there is a search for locally adapted strains of entomopathogenic (bacteria, viruses, fungi) for effective management of insect pests for that particular environment. In many cases successful control of insects have been achieved by using local strains rather than exotic microorganisms (strains). Epizootic of disease by entomopathogenic fungi *Beauveria* spp. have been reported as on *S. litura* in India (Ranga Swami *et al*., 1968; Zaz and Kushwaha, 1983) [48, 63]. So, it is assumed that local strains of microorganisms might have well adapted to that particular environment from where they are isolated and may play major role. In this study, the growth parameters of fungal isolates in each of *Beauveria* spp. and in-vitro bio-efficacy with selected commercial entomopathogenic fungal formulations and local strains isolated against *Spodoptera litura* was demonstrated. Chemical pesticides being synergistic/antagonistic among themselves, may have antagonistic or synergistic effects on the potentiality of *Beauveria* spp. and may influence natural epizootics. Such situation warrants only the compatible insecticides and/or fungicides to be used in combination with these microbial agents to derive the fullest potential of the organism with least environmental pollution along with cost effectiveness. Therefore, for successful establishment of entomopathogenic fungi in IPM programmes, its compatibility with insecticides and fungicides is very important to manage the insect pests.

Material and Methods
Compatibility of selected entomopathogenic fungal isolates of *B. bassiana* (Bb-L-2) with selected insecticides
The commonly used insecticides were tested *In vitro* for their inhibitory effect, if any on selected entomopathogenic fungal isolates of *B. bassiana* in terms of radial growth following Poison Food Technique (Nene and Thapliyal, 1993) [53, 55]. The insecticides and their concentrations tested in this experiment are listed in the table below. Each test insecticide at field recommended concentration was tested with five replications.

Incorporation of test insecticides into the media

Sterilized SDAY medium was melted and cooled but before solidification, the test insecticides at field recommended concentration, Indoxacarb-0.0045 percent, Spinosad-0.018 percent, Monocrotophos-0.310 percent, Chlorpyriphos-0.050 percent, Diclorovos (DDVP)-0.120 percent, Endosulfan - 0.350 percent, Neemoil-5 percent were added treatment wise by using micropipette. The medium was shaken vigorously for even mixing of the contents and poured into sterile petriplates of 9.5 x 1.5 cm. hundred ml medium was poured evenly in five plates and allowed to solidify for further tests.

Inoculation of the medium with entomopathogenic fungal isolates of *B. bassiana* (Bb-L-2) mycelial mat

Circular discs of 10 mm diameter were cut from vigorously grown culture of B. *bassiana* using a sterile cork borer and such discs were placed in the middle of each petriplate on the medium mixed with insecticide. Medium inoculated with the fungus without insecticide served as untreated control. These steps were carried out under aseptic conditions inside an inoculation chamber sterilized with UV radiation. These plates were incubated at 25 $\pm$ 1ºC for 10 days.

Radial growth of the fungus was measured after 10 days and compared with untreated control. The number of conidia per unit area and viability of conidia were also recorded following the procedures mentioned in earlier experiment and compared with untreated control using the formula.

$$R = \frac{C - T}{C} \times 100$$

Where,

R = Per cent reduction of radial growth / conidia per unit area / conidial viability.

C = Radial growth / conidia per unit area / Conidial viability of fungi grown on control or untreated medium.

T = Radial growth / conidia per unit area / conidial viability of fungi grown on insecticide treated medium.

Joint action of microbial, botanical and chemical insecticides with selected fungal isolate of *B. bassiana* (Bb-L-2).

Isolation, purification, mass multiplication and maintenance of Sl. NPV

Spodoptera NPV cultures were obtained from Project Directorate of Biological Control (P.D.B.C.), Bangalore. Mass multiplication of nuclear polyhedrosis virus was done on larvae of *S. litura*. Castor leaf dipped in viral suspension of 1 x 10^6 PIBs/ml were fed to the third instar larvae of *S. litura* for 24 hours, later transferred to fresh semi-synthetic diet individually in glass vials. Larvae were reared on diet till development of disease.

Diseased larvae were collected and stored in distilled water in 100 ml of conical flask and allowed to putrefy for 15 days. The putrefied larvae were macerated using the glass rod and then filtered through double layer muslin cloth twice. The PIBs were purified by alternate cycle of low (250-500 g rpm for 5-10 min) and high (5000-7000 g rpm for 30-60 min) speed centrifugation. The PIBs was stored at 4 ºC in the refrigerator for further use.

Mass Multiplication of standard isolate of *Bacillus thuringiensis* (HD-1)

The bacterial culture (HD-1) was collected from the Department of Plant Protection, Sam Higginbottom Institute of Agriculture Technology and sciences, Allahabad

Bacillus thuringiensis easily produced on artificial media by adopting conventional fermentation techniques either on surface or semi solid fermentation or submerged fermentation. strains from diseased insects is isolated as follow, the non-spore forming bacteria are eliminated by heating 60˚C for 50 minutes, the growth of other spore forming is reduced by addition of 50µg/ml polymedium B to the nutrient agar medium. The slant culture of pure *Bacillus thuringiensis* (HD-1) is transferred into 300ml of polymedium (Pepton 0.5%, Glycerol 1%, Yeast extract 1%, Beef extract 0.5% and NaCl 0.3% with pH adjusted to 7.2) in 500ml flask. The incubation is done at 32˚C in an incubation shaker for 48hrs. Once the incubation is completed the whole content is transferred to fermentor of 15 litres capacity containing 10 liter of presterilized poly-media. The inoculated medium is incubated in the fermentor for 72hrs. for further sealing of production 15 litre of growing culture is added to 300 litre capacity fermentor. The spore count of the fermented liquor should be 2.5×10^9 spore/ml. The content of each fermented aseptically centrifuged at 5000 rpm for 10-15 minutes. The sedimentation is washed 3 times with distilled water and transferred to small quantity of polymedium, vacuum dried and concentrated into powder and then packed Carozzi *et al*., (1991).

Preparation of Neem seed kernel extract (NSKE-5%)

Fifty grams of neem seeds were shade dried, crushed and then soaked overnight in little quantity of water. Later, the mixture was squeezed through muslin cloth and the volume was made upto one liter so as to obtain 5% solution.

The tests were conducted on the third instar larvae of *S. litura* using LC_{50} and LC_{25} combinations of entomopathogens and recommended dose and half recommended dose of neem oil, NSKE and spinosad (45 SC) (Table 2).

Fungus (*B. bassiana* (Bb-L-2) and *M. anisopliae* (Ma-L-1)) and *Bacillus thuringiensis* (HD-1)

Larvae of uniform age and size from the laboratory cultures were used for this study. Four combinations of concentrations were used for infecting the larvae. Healthy, third instar larvae were sprayed with conidial suspension and later, the larvae were allowed to feed on leaf treated with desired concentration of *Bacillus thuringiensis*. Observation on per cent mortality was observed daily up to ten days after the treatment.

Fungus ((*B. bassiana* (Bb-L-2) and *Sl* NPV

The respective conidial suspensions were sprayed on third instar larvae of *S litura* and later allowed the larvae to feed on leaf dipped in desirable concentration of NPV. Four combination of concentration were tried using effective lethal concentration, LC_{50} and LC_{25}.

Fungus *B. bassiana* (Bb-L-2) with neem oil, NSKE and spinosad (45 SC)

The respective conidial suspensions and insecticides were sprayed on third instar larvae of *S. litura*.

The following formula was used to determine expected mortality, if the two pathogens acted independently of each other.

$$E = (Ob + Os)-((Ob\times Os) / 100))$$

Where,
E = per cent expected mortality.
Ob = Observed percentage mortality produced by one pathogen.
Os = Observed percentage mortality produced by another pathogen.
Chi-square test
$X^2 = (Oc - E)^2 / E$

Where,
Oc = Observed percentage mortality from the combination
E = per cent expected value

The calculated Chi-square value were compared to the Chi-square table value for 1 degree of freedom P=0.05. If the table value exceeded the calculated, it was concluded that the observed mortality for the combination of pathogen was within the range expected from an additive effect. If the calculated value exceeded the table value, a synergistic reaction between the pathogen was suspected (Finney, 1964) [21].

Results and Discussion

In vitro compatibility of selected entomopathogenic fungi *B. bassiana* (Bb-L-2) with botanicals and chemical insecticides against *Spodoptera litura.*

Effect of selected insecticides on radial growth of the selected test strains of *B.bassiana* (Bb-L-2) toxic effect of six insecticides *viz.* indoxacarb, spinosad, monocrotophos, chlorpyriphos, diclorovos and endosulfan and two neem formulations was tested on the radial growth. Significant reduction in radial growth of Bb-L-2 compared with control (5.46 cm) was not observed due to indoxacarb and spinosad which recorded 5.37 and 5.44 cm of radial growth, respectively, while monocrotophos, chlorpyriphos, diclorovos and endosulfan recorded *viz.*1.55, 1.39, 0 and 1.85 cm radial growth, respectively, with 62.73, 72.40, 100 and 60.83 per cent growth reduction, respectively, radial growth which was significantly lower compared to control. While diclorovos brought out 100 per cent growth reduction in compared to control. Results of evaluation of neem based pesticides revealed that these pesticides were comparatively safer for this bio-agent. Neem oil affected the radial growth (20.18% reduction) while NSKE effected the radial growth (18.35%) (Table 1). Similarly, Gupta *et al.* (2002) [26] reported that higher concentrations of neem formulations were also compatible to *B.bassiana* under *In vitro* conditions (Table 1). Introduce multiple mortality factors against the target pest with insecticide making the insect physiology weak to a desired degree which makes it much more susceptible to the attack of the entomopathogens (Fedorinehik, 1974) [17] and also delay the chances of expression of resistance to new insecticides (Georghiou, 1983) [25]. This approach in pest management was explored by Steinkraus (1996) [52] and Brown *et al.* (1997) [8] who found that the combination of imidacloprid and *B. bassiana*. The inhibition of growth and sporulation might be due to the interference of chlorpyriphos in the uptake of carbohydrates and nitrogen from exogenous source (media) which are essential for growth and sporulation of entomopathogenic fungi (Pachamuthu *et al*., 1999) [4]. Ambethgar *et al.* (2009) [2] reported that *B.bassiana* exhibited 75.55, 100.00, 100.00, 48.33, 60.74, 54.44, 59.66, 70.33, 47.41, 59.66, 48.88 and 41.48 per cent inhibition, respectively to insecticides at normal field recommended dose *viz.* acephate (1.0%), carbaryl(2.0%), carbofuran (1.0%), chloropyriphos (2.5%), dichlorophos (1.5%), dimethoate (2.0%), endosulfan (2.0%), fenthion (1.0%), monocrotophos (2.0%), phosalone (2.0%), phosphamidon (1.0%) and quinolphos (2.0%) and 10 time higher rate of insecticides exhibited 100% inhibition rate of fungus growth, however, at 2.5% concentration, neem oil and NSKE exhibited 36.30% and 22.22% mycelial inhibition, respectively. Haseeb, (2009a) [27] revealed that the growth of test fungus strongly inhibited by insecticides by insecticides in descending order were chloropyriphos, endosulfan, malathion, methyl parathion, monocrotophos and fenvelerate (72.7-94.6% reduction in growth dia.) while dimethoate affected growth 32.3% reduction and compatibility of six strains of *B.bassiana* with four commonly used insecticides, *viz.*, imidacloprid, spinosad, indoxacarb and chlorpyriphos. All the strains were compatible with imidacloprid, spinosad and indoxacarb. Chlorpyriphos was found to be highly incompatible with all the strains of *B.bassiana* and exhibited high inhibition of growth (Rajanikanth *et al*. 2010) [47].

Table 1: *In vitro* compatibility of *B. bassiana* (Bb-L-2) strain with selected insecticides

Insecticides	Concentration (%)	Radial growth (cm) after 10 days	Per cent inhibition over control
Indoxacarb	0.0045	5.37 ^{c}	1.68
Spinosad	0.018	5.44 ^{b}	0.29
Monocrotophos	0.310	1.55 ^{g}	71.65
Chlorpyriphos	0.050	1.39 ^{h}	74.39
Diclorovos (DDVP)	0.120	0 ^{i}	100
Endosulfan	0.350	1.85 ^{f}	66.15
Neem oil	5	3.45 ^{e}	36.81
NSKE	5	3.97 ^{d}	27.29
Control	-	5.46 ^{a}	0
SE(m) ±		0.027	-
CD (0.01)		0.077	-

Figures indicated by same letters are not significantly different from one another as per DMRT

In vitro compatibility of selected entomopathogenic fungi *B. bassiana* (Bb-L-2) with botanicals and chemical insecticides against *Spodoptera litura.*

Joint action of *B. bassiana* (Bb-L-2) and Spodoptera. NPV on third larval instar of *S. litura*

At the higher dosage levels tested (LC_{50}) *B. bassiana* (Bb-L-2) and *Sl.* NPV individually caused 45.44 per cent and 57.33 per cent mortality. At lower dosage (LC_{25}) *B. bassiana* (Bb-L-2) and *Sl.* NPV gave 27.08 percent and 27.61 per cent mortality, respectively (Table 2 &3).

Table 2: Percent mortality of *S. litura* larvae observed at different concentrations of selected entomopathogens and insecticides.

Insecticides	Dose	% Mean mortality (after 10 days)
Beauveria bassiana (Bb-L-2)	LC_{50}	45.44
	LC_{25}	27.08
Metarhizium anisopliae (Ma-L-1)	LC_{50}	47.91
	LC_{25}	20.14
Bacillus thuringiensis (HD-1)	LC_{50}	53.33
	LC_{25}	25.66
Spodoptera NPV	LC_{50}	57.33
	LC_{25}	27.61
Spinosad (Tracer 45 SC)	RD (Recommended dose)	94.52
	1/2 RD (Recommended dose)	68.31
Neem oil	RD (Recommended dose)	34.27
	1/2 RD (Recommended dose)	20.18
NSKE (Neem seed kernal extract)	RD (Recommended dose)	29.33
	1/2 RD (Recommended dose)	17.83
B. bassiana (Bb-L-2): $LC_{50} = 5.0 \times 10^6$ conidia/ml & $LC_{25} = 2.0 \times 10^4$ conidia/ml *M.* anisopliae (Ma-L-1): $LC_{50} = 1.6 \times 10^6$ conidia/ml & $LC_{25} = 1.2 \times 10^4$ conidia/ml *Bacillus thuringiensis* (HD-1): $LC_{50} = 3.5 \times 10^4$ spore/ml & $LC_{25} = 2.9 \times 10^3$ spore/ml *Spodoptera NPV:* $LC_{50} = 4.2 \times 10^4$ PIBs/ml & $LC_{25} = 3.3 \times 10^3$ PIBs/ml Spinosad (Tracer 45 SC): RD (Recommended dose)=0.018% & 1/2 RD (Recommended dose)=0.009% Neem oil: RD (Recommended dose)=5% & 1/2 RD (Recommended dose)=2.5% NSKE (Neem seed kernal extract): RD (Recommended dose)=5% & 1/2 RD (Recommended dose)=2.5%		

Table 3: Joint action of *B. bassiana* (Bb-L-2) and *Sl.* NPV on third larval instar of *S. litura*

Combination *B. bassiana* (Bb-L-2) + *Sl.* NPV (Concentration)	Expected per cent mortality	Per cent mortality observed	Chi - Square
$LC_{50} + LC_{50}$	76.75	65.15	2.22
$LC_{25} + LC_{50}$	60.50	49.20	2.18
$LC_{50} + LC_{25}$	68.88	58.07	1.72
$LC_{25} + LC_{25}$	47.21	38	1.79
Spodoptera NPV: $LC_{50} = 4.2 \times 10^4$ PIBs/ml & $LC_{25} = 3.3 \times 10^3$ PIBs/ml *B. bassiana* (Bb-L-2): $LC_{50} = 5.0 \times 10^6$ conidia/ml & $LC_{25} = 2.0 \times 10^4$ conidia/ml			

A combination of both at LC_{50} resulted in 65.15 per cent mortality, which is less than the mortality expected from such combination. Combination of LC_{50} of *B. bassiana* (Bb-L-2) and LC_{25} of *Sl.* NPV and vice versa, resulted in 49.20 per cent and 58.07 per cent mortality, respectively. At lower dosage (LC_{25} + LC_{25}) the same combination gave 38 per cent mortality which was less than the mortality expected from such combination (Table 3). Simultaneous exposure of *B. bassiana* (Bb-L-2) and *B. thuringiensis* (HD-1) against the test species at LC_{50} each resulted in 61.81 per cent mortality. The expected mortality for same combination was 74.54 per cent (Table 26). Mixture of LC_{50} of *B. bassiana* (Bb-L-2) and LC_{25} of *B. thuringiensis* (HD-1) and vice versa showed 47.92 per cent and 54.86 per cent mortality. At LC_{25} same combination gave 37.41 per cent mortality and the expected mortality from such combination was45.79 per cent (Table 5).

Table 4: Joint action of *Sl.* NPV and *B. thuringiensis* (HD-1) on third larval instar of *S. litura*

Combination *Spodoptera* NPV + *B. thuringiensis* (HD-1) (Concentration)	Expected per cent mortality	Per cent mortality observed	Chi - Square
$LC_{50} + LC_{50}$	80.09	65.57	2.28
$LC_{50} + LC_{25}$	68.29	63.33	0.36
$LC_{25} + LC_{50}$	66.22	54.45	2.09
$LC_{25} + LC_{25}$	46.18	35.26	2.58
Spodoptera NPV: $LC_{50} = 4.2 \times 10^4$ PIBs/ml & $LC_{25} = 3.3 \times 10^3$ PIBs/ml *Bacillus thuringiensis* (HD-1) : $LC_{50} = 3.5 \times 10^4$ spore/ml & $LC_{25} = 2.9 \times 10^3$ spore/ml			

Table 5: Joint action of *B. bassiana* (Bb-L-2) and *B. thuringiensis* (HD-1) on *S. litura*

Combination *B. bassiana* (Bb-L-2) + *B. thuringiensis* (HD-1) (Concentration)	Expected per cent mortality	Per cent mortality observed	Chi - Square
$LC_{50} + LC_{50}$	74.54	61.81	2.17
$LC_{25} + LC_{50}$	59.44	47.92	1.53
$LC_{50} + LC_{25}$	65.97	54.86	1.87
$LC_{25} + LC_{25}$	45.79	37.41	2.01
Bacillus thuringiensis (HD-1): $LC_{50} = 3.5 \times 10^4$ spore/ml & $LC_{25} = 2.9 \times 10^3$ spore/ml *B. bassiana* (Bb-L-2): $LC_{50} = 5.0 \times 10^6$ conidia/ml & $LC_{25} = 2.0 \times 10^4$ conidia/ml			

Joint action of *B. bassiana* (Bb-L-2) and selected insecticides on third larval instar of *S. litura*

The *B. bassiana* (Bb-L-2) tested at LC_{50} and LC_{25} dosage and spinosad tested at recommended dose and half recommended dose individually caused 45.44, 27.08, 94.52 and 68.31 per cent mortality and simultaneous exposure of *B. bassiana* (Bb-

L-2) and spinosad against the test insect with four different dose combinations (LC_{50} dose of *B. bassiana* (Bb-L-2) and recommended dose of spinosad, LC_{50} dose of *B. bassiana* (Bb-L-2) and half recommended dose of spinosad, LC_{25} dose of *B. bassiana* (Bb-L-2) and recommended dose of spinosad, LC_{25} dose of *B. bassiana* (Bb-L-2) and half recommended dose of spinosad) showed 100 per cent mortality with four different dose combinations (Table 6). The *B. bassiana* (Bb-L-2) tested at LC_{50} and LC_{25} dosage and neem oil tested at recommended dose and half recommended dose individually caused 45.44, 27.08, 34.27 and 20.81 per cent mortality and simultaneous exposure of *B. bassiana* (Bb-L-2) and neem oil against the test insect with four different dose combinations (LC_{50} dose of *B. bassiana* (Bb-L-2) and recommended dose of neem oil, LC_{50} dose of *B. bassiana* (Bb-L-2) and half recommended dose of neem oil, LC_{25} dose of *B. bassiana* (Bb-L-2) and recommended dose of neem oil, LC_{25} dose of *B. bassiana* (Bb-L-2) and half recommended dose of neem oil) showed 71.33, 61.08, 57.00 and 35.17 per cent mortality with four different dose combinations respectively (Table 7).

The *B. bassiana* (Bb-L-2) tested at LC_{50} and LC_{25} dosage and NSKE tested at recommended dose and half recommended dose individually caused 45.44, 27.08, 34.27, 29.33 and 17.83 per cent mortality and simultaneous exposure of *B. bassiana* (Bb-L-2) and NSKE against the test insect with four different dose combinations (LC_{50} dose of *B. bassiana* (Bb-L-2) and recommended dose of NSKE, LC_{50} dose of *B. bassiana* (Bb-L-2) and half recommended dose of NSKE LC_{25} dose of *B. bassiana* (Bb-L-2) and recommended dose of NSKE, LC_{25} dose of *B. bassiana* (Bb-L-2) and half recommended dose of NSKE) showed 46.81, 44.31, 39.27 and 32.78 per cent mortality with four different dose combinations respectively (Table 8). Similar findings of synergism of microorganism and chemical insecticides have been very well documented (Benz, 1971 [5]; Ferron, 1978 [18, 19]; Hukuhara *et al.* (1987) and Wang *et al.* (1994) [60]. Similar findings of compatibility of insecticides with various entomopathogens was reported by Fargues (1975) [15] who reported that combinations of sublethal amount of insecticides were clearly compatible with *B. bassiana* and use of mixtures might have advantages for Colorado potato beetle management and Georghiou (1983) [25] reported that insecticide-pathogen combinations introduce multiple mortality factors against the pest and increasing the number of mortality factors used against insect and should delay any expression of resistance to new insecticides. Anderson *et al.* (1989) [3] reported that in bioassay with neonate of Colorado potato beetle, effects *B. bassiana* alone were extremely variable and combination of *B.bassiana* with insecticides *viz.*Thuringiensin, Abamectin and Triflamuron were consistently more toxic than *B. bassiana* and Sinha (1993b) [51], reported that a water dispersible powder formulation of neem product (Achook) checked the larval and pupal survival and growth and adult emergence of *H. armigera.* and Ingle *et al.* (2008) [28] reported that effectiveness of entomogenous fungus, *Nomuraea rileyii* with combination of different plant oils on chickpea against *Helicoverpa armigera*, sprayings of soybean and sunflower oil formulation combinations were found very effective in reducing larval population, pod damage and increase in grain yield of chickpea.

Table 6: Joint action of *B. bassiana* (Bb-L-2) and Spinosad on third larval instar of *S. litura*

Combination *B. bassiana* (Bb-L-2) + Spinosad (Tracer 45 SC) (Concentration)	Per cent mortality observed
LC_{50} + R.D	100
LC_{50} + ½ R.D	100
$LC_{2\,5}$ +R.D	100
LC_{25} + ½ R.D	100
B. bassiana (Bb-L-2): LC_{50} = 5.0 x 10^6 conidia/ml & LC_{25} =2.0 x 10^4 conidia/ml Spinosad (Tracer 45 SC): RD =0.018% & 1/2 RD (Recommended dose)=0.009%	

Table 7: Joint action of *B. bassiana* (Bb-L-2) and Neem oil on *S. litura*

Combination *B. bassiana* (Bb-L-2) + Neem oil (Concentration)	Per cent mortality observed
LC_{50} + R.D	71.32
LC_{50} + ½ R.D	61.08
LC_{25} + R.D	57.00
LC_{25} + ½ R.D	35.17
B. bassiana (Bb-L-2): LC_{50} = 5.0 x 10^6 conidia/ml & LC_{25} =2.0 x 10^4 conidia/ml Neem oil: RD (Recommended dose)=5% & 1/2 RD (Recommended dose)=2.5%	

Table 8: Joint action of *B. bassiana* (Bb-L-2) and NSKE on third larval instar of *S. litura*

Combination *B. bassiana* (Bb-L-2) + NSKE (Concentration	Per cent mortality observed
LC_{50} + R.D	46.81
LC_{50} + ½ R.D	44.31
LC_{25} + R.D	39.27
LC_{25} + ½ R.D	32.78
B. bassiana (Bb-L-2): LC_{50} = 5.0 x 10^6 conidia/ml & LC_{25} =2.0 x 10^4 conidia/ml NSKE (Neem seed kernal extract): RD=5% & 1/2 RD (Recommended dose)=2.5%	

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