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Bio-efficacy of some entomopathogens as well as newer molecule of insecticides against tomato fruit borer *Helicoverpa armigera* (Hubner)

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Abstract
Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and commercially important vegetable crops in India. Among many factors responsible for low yields of tomato, insect pests are major ones that have been reported to attack tomato at all stages of crop growth. The damage caused by fruit borer, *Helicoverpa armigera* (Hubner) surpass the loss caused by all other insect pests together and it has been reported that the loss due to this pest ranges from 20 to 50 per cent. To address the issue an experiment were taken up where three entomopathogens were evaluated against *Helicoverpa armigera* to generate information regarding LC 50 and LT 50 along with five newer molecules. Observation data indicates the supreme toxicity of Emamectin benzoate among all the treatments as its shows lowest LC50 values on 12 and 24 hours are0.37 x 10^{-4} and 0.11 x 10^{-4} ml/l, respectively. Whereas, among microbials *B. thuringiensis* displayed lowest LC 50 values i.e, 5.0 x 10^{8}, 0.58 x 10^{8} and 0.12 x 10^{8} IU/gm, respectively, in 96, 120 and 144 hours. In terms of Median lethal time (LT50) was estimated for Emmamectin Benzoate (8.81 hour) was observed lowest followed by Cyantraniliprole (15.09 hour), Chlorantraniliprole (35.54 hour), Flubendiamide (44.41 hour), Spinosad (44.62 hour), *B. thuringiensis* (83.72 hour), HaNPV (114.58 hour) and *B. bassiana* (154.63 hour). This result is good evident of potency of newer molecules against *H. armigera* and it is well established that entomopathogens also has moderate level of efficacy which have no adverse effect on environment.

Keywords: entomopathogen, *Helicoverpa armigera*, microbial, newer molecule

Introduction
The main constraints to tomato production in the country are diseases and insect pests. The fruit borer, *Helicoverpa armigera* is a notorious pest which [2] considered as one of the major pests of tomato, inflicting devastating crop losses in India [3]. Recently, this pest has attained the status of a national pest [4]. As the status of pest is major in India, the management of fruit borer through. Due to rapid effect, ease of application and availability of chemical insecticides, adoption by farmers is very high and indispensable uses leads to inevitable undesirable side effects. Unavoidably high level of pesticide residues which may be highly hazardous causing serious problems including pest resistance, pest outbreak, pest resurgence and environmental pollution [5]. As researchers concentrating more on non-chemical solution for the pest menace, where entomopathogens emerged as very effective option. The concept of Integrated Pest Management (IPM) is becoming a practicable and acceptable approach over the world. The incorporation of chemicals and entomopathogens in IPM strategy would be very effective and safe approach. Hence, keeping the above view in mind an experiment executed where emphasis were given to evaluate some microbials as well as newer molecule of insecticides against *H. armigera* in the laboratory.

Materials and Methods
Nucleus culture
Experiment conducted in the laboratory condition in the Department of Plant Protection, Palli Siksha Bhavana, Visva Bharati. Tomato var. *Patharkuchi* (local variety) was cultivated in farmer's field near Sriniketan of Birbhum district of red lateritic zone of West Bengal. The infested fruits with *H. armigera* collected from the field and reared in the laboratory. Filled collected larvae kept in containers individually (2.5 cm diam.x10 cm long) till adult emergence. Fresh sliced unripe tomato was offered to insect culture. The larvae were gently taken out with the help of a fine camel brush during the food change and placed them on the

fresh foods. Before pupation each container filled up with sterilized soil at the base. After adult emergence the insect was paired separately in different glass jars (6″ diam. and 10″ long) lined with papers on inner wall with muslin cloths on the tops. Cotton swab soaked in 10% honey solution provided as food for the adults. After oviposition, eggs were collected with fine camel brush and placed initially into a petridish (4 cm diam.). After hatching larvae were reared separately and third instar *H. armigera* larvae were used for the experiment.

Bioassay with entomopathogens and newer molecule of insecticides

Two hour pre starved third instar *H. armigera* larvae taken from nucleus culture for conducting experiments. For bioassay, nine treatments including one control collected from different sources have been presented in Table 1. The treatments were replicated four times. Each treatment has four concentrations prepared from serial dilution, where the recommended dose was taken as highest concentration. One ml solution of each concentration was sprayed on both upper and lower surfaces of tomato leaves using Potter's tower at 15 psi. Single number of larvae were released individually on the treated leaves for each concentration and kept individually in separate plastic vials (2.5 cm diam. x 10 cm) after spraying. On the petiole of treated tomato leaves 1 per cent agar media were dispensed which fixed using parafilm. Petiole was pierced out from inside out the container. Agar media retained turgidity of leaves for 72 hrs. After which fresh leaves were offered using similar technique. Observations for larval mortality were taken at 12 h interval for chemical insecticides and 24 h for microbials. Observations on each treatment ended when 100% mortality occurred or pupation started. The median lethal concentrations (LC50) and median lethal times (LT50) of each treatment were calculated using probit analysis [6].

Table 1: Treatment details

Treatments	Company Name/Source	Recommended dose	Concentrations			
T1 *Beauveria bassiana* (10^9 spores/gm)	Vivekananda Institute of Biotechnology, Nimpith, South 24 pgs.	10^9 spores/gm	10^9	10^8	10^7	10^6
T2 *Bacillus thuringiensis* (10^9 IU/gm)	Vivekananda Institute of Biotechnology, Nimpith, South 24 pgs	10^9 IU/gm	10^9	10^8	10^7	10^6
T3 HaNPV (10^9 POB/ml)	Vivekananda Institute of Biotechnology, Nimpith, South 24 pgs	10^9 POB/ml	10^9	10^8	10^7	10^6
T4 Emmamectin Benzoate 5 SG	Dhanuka agrotech	0.5 ml/l	0.5	0.25	0.125	0.062
T5 Spinosad 45 SC	Bayer crop science	0.3 ml/l	0.3	0.15	0.075	0.037
T6 Flubendiamide 480 SC	Bayer crop science	0.2 ml/l	0.2	0.1	0.05	0.025
T7 Chlorantraniliprole 20 SC	Dupont	0.3 ml/l	0.3	0.15	0.075	0.037
T8: Cyantraniliprole 10.26 OD	Dupont	1 ml/l	1	0.5	0.25	0.125

T9 Control (Water spray)

Units of different concentrations are same as recommended dose.

Results and Discussion

For understanding the effectivity of different newer molecules as well as entomopathogens against *H. armigera* an experiment was conducted in the laboratory. LC50 values at different exposure periods were calculated along with their fiducial limits. The amount of concentration required for killing the 50% population of test insects in different hour (LC50) during the experimental period presented in Table 2. Median lethal concentrations (LC50) were calculated by probit analysis for all the microbials as well as chemical insecticides in different set of time. Three microbials were used for the experiment, where LC50 values of *B. bassiana* were calculated as 1.4 x 10^8spores/gm, 0.15 x 10^8 spores/gm and 0.03 x 10^8 spores/gm in 192, 216 and 240 hours, respectively. In the year 2007 an experiment conducted by Elanchezhyan *et al.* [7]. Observed efficacy of *B. bassiana* against fruit borer *H. armigera*. While, the LC 50 value for *B. thuringiensis* were 5.0 x 10^8, 0.58 x 10^8 and 0.12 x 10^8 IU/gm, respectively, in 96, 120 and 144 hours. Whereas, median lethal concentrations for Ha NPV were calculated as 6.3 x 10^8 POB/ml (144 hours), 0.45 x 10^8 POB/ml (168 hours) and 0.17 x 10^8 POB/ml (192 hours). Later on in Israel, applications of *B. thuringiensis* reported to suppress population of *H. armigera* [8]. Sonalkar *et al*. (1998) [9] also observed similar finding and revealed that *H. armigera* can be controlled successfully using NPV.

In this set of experiment 5 insecticides were taken viz. Emmamectin Benzoate 5 SG, Spinosad 45 SC, Flubendiamide 480 SC, Chlorantraniliprole 20 SC and Cyantraniliprole 10.26 OD. The LC50 values were calculated at different set of time. On 12 and 24 hours, LC50 values of Emamectin Benzoate were 0.37 x 10^{-4} and 0.11 x 10^{-4} ml/l, respectively. Similarly Jansson *et al.* (1997) [10] reported very low LC50 and LC90 values of emamectin benzoate (0.04 and 0.006 mg/ ml, respectively) against *Helicoverpa virescens*. However, Lopez *et al*. (1997) [11] reported a higher LC50 (< 5.0 ppm) against *Helicoverpa zea*. But Dunbar *et al.* (1998) [12] stated that emamectin benzoate was highly toxic to lepidopterous pests with LC90 values ranging between 0.001 and 0.02 mg/ml in ingestion based foliar spray assays for tobacco budworm.

Spinosad also exerted mortality to half of the test insect population with the concentrations 0.27 x 10^{-4}, 0.086 x 10^{-4}, 0.052 x 10^{-4} and 0.041 x 10^{-4} ml/l on 48, 60, 72 and 84 hours, respectively. Whereas, LC50 values of Flubendiamide were 1.4 x 10^{-4} (48 hours), 0.29 x 10^{-4} (60 hours) and 0.14 x 10^{-4} (72 hours) ml/l. Kubendran *et al.* (2008) [13] found that the toxicity (LD50) of flubendiamide was relatively less variable falling within a range of 0.130-0.127 mg/larva, where this experiment produced fiducial limit of flubendiamide 0.084 x 10^{-4}- 0.46 x 10^{-4} ml per litre against *H. armigera* at 60th hour after treatment. Lahm *et al.* (2005) [14] reported that LC50 of Chlorantraniliprole against a broad range of lepidopteran pests including *P. xylostella, S. frugiperda* and *H. virescens* ranged between from 0.01 to 0.03 ppm. Similar way, in this experiment also Chlorantraniliprole also found effective against lepidopteran pest i.e, *H. armigera*. On 48, 60 and 72 hours the LC50 values of Chlorantraniliprole were 0.17 x 10^{-4}, 0.087 x 10^{-4} and 0.054 x 10^{-4} ml/l, respectively. The LC50 values on 24, 36 and 48 hours of exposure were also calculated separately for Cyantraniliprole 10.26% OD that had the LC50 values of 4.9 x 10^4, 2.7 x 10^{-4} and 2.1 x 10^{-4} ml/l, respectively. In a laboratory experiment, LC50 value of chlorantraniliprole (0.1 ppm) found significantly lower as compared to other two standard insecticides such as

indoxacarb (1.5 ppm) and cypermethrin (13.5 ppm) in an insecticide treated diet assay against *H. virescens* [15]. In another study, Temple *et al*. (2009) [16] observed that LC50 and LC90 of chlorantraniliprole for the field and laboratory populations of *H. virescense* were 0.03 and 0.22 ppm for the field and 0.02 and 0.13 ppm for laboratory populations respectively, while LC50 and LC90 for *H. armigera* values ranging from 0.04-0.09 ppm and 0.11-0.34 ppm, respectively by incorporated artificial diet assay. The LC50 value of chlorantraniliprole estimated 0.0731 mg/ml against resistant strain and 0.0954 mg/ml for susceptible strain of *H. armigera* [17]. Zhang *et al.* (2013) [18] reported that the susceptibility of third instars larvae of *H. armigera* to chlorantraniliprole analyzed by incorporated artificial diet assay, resulted in LC10, LC20, LC40, and LC50 values of 3.790, 7.978, 21.577, and 33.121 mg/L, respectively.

Median lethal time (LT50) was estimated for each insecticide at recommended field doses which is presented in Table 3. At recommended doses the LT50 value of Emmamectin Benzoate (8.81 hour) was observed lowest followed by Cyantraniliprole (15.09 hour), Chlorantraniliprole (35.54 hour), Flubendiamide (44.41 hour), Spinosad (44.62 hour), *B. thuringiensis* (83.72 hour), HaNPV (114.58 hour) and *B. bassiana* (154.63 hour). Similarly, Khan *et al*. (2010) [19] reported that spinosad (300 ppm) and indoxacarb (200 ppm) were highly toxic to first and second instars larvae of *H. armigera* which could give complete mortality at 48 h after ingestion based on leaf dip assay. After conducting another experiment by Prasad *et al*. in 2010 [20], where researchers studied four different concentrations of *B. bassiana* on the 4th instar larve of *H. armigera* and recorded the larval mortality two days after application with the highest mortality (76.7%) at highest dose of 0.25 ml having10^8 spores/ml. In another studies Ganguli *et al.* (1997) [21] concluded that spraying with NPV (250 LE/ha) at the time of the appearance of the pest, followed by 7 days later by endosulfan at 0.035% protected the tomato crop from *H. armigera*. The bio-assay study inferred that all the microbials and new generation of insecticides proved effective against tomato fruit borer larvae.

Conclusion

Experiment findings indicates that entomopathogens has efficacy against *H. armigera*, along with that it is well understood that selected chemical insecticides proved effective against *H. armigera.* In terms of median lethal concentrations of different treatments, all the chemical insecticides proved very much toxic as its shows very low LC 50, whereas all the microbial gave much higher level of LC 50 that indicates trends of lesser toxicity. All the chemical insecticides showed LT 50 within 48 hours of treatment, but all the microbial gave the LT 50 beyond that time line. For better understanding the efficacy in field, experiment can be taken up to see individual effect as well as combined effect. Also it will be interesting to see the efficacy of both segments i.e, microbials and chemicals can be applied in following weeks. That way uses of chemical will be less and at the same point pest mortality can be expected.

Table 2: Median lethal concentrations of different treatments against *H. armigera*

Treatment	Hour	LC_{50}	Fiducial limit (p=0.05)	χ^2	Reg. Equation
T_1 : *Beauveria bassiana* (spores/gm)	192	1.4×10^8	0.40×10^8 - 10×10^8	1.2	Y=1.40 + 0.44x
	216	0.15×10^8	0.03×10^8 - 0.55×10^8	0.36	Y=1.90 + 0.43x
	240	0.03×10^8	0.005×10^8 - 8.9×10^8	7.78*	Y=1.57 + 0.54x
T_2 : *Bacillus thuringiensis* (IU/gm)	96	5.0×10^8	4.7×10^8 - 58×10^8	0.78	Y= 0.61 + 0.50x
	120	0.58×10^8	0.21×10^8 - 1.9×10^8	1.11	Y= 0.66 + 0.56x
	144	0.12×10^8	0.002×10^8 - 6.9×10^8	9.22*	Y= 0.09 + 0.69x
T_3 : HaNPV (POB/ml)	144	6.3×10^8	2.0×10^8 - 26×10^8	0.86	Y= 0.69 + 0.49x
	168	0.45×10^8	0.08×10^8- 1.3×10^8	1.0	Y= 0.85 + 0.54x
	192	0.17×10^8	0.02×10^8- 0.47×10^8	3.8*	Y= -0.05 + 0.70x
T_4 : Emmamectin Benzoate (ml/lt)	12	0.37×10^{-4}	0.26×10^{-4} - 0.71×10^{-4}	0.10	Y= 5.7 + 1.7x
	24	0.11×10^{-4}	0.083×10^{-4} - 0.14×10^{-4}	5.9*	Y= 7.8 + 2.9x
T_5 : Spinosad (ml/lt)	48	0.27×10^{-4}	0.18×10^{-4} - 0.76×10^{-4}	0.36	Y= 5.8 + 1.5x
	60	0.086×10^{-4}	0.056×10^{-4} - 0.12×10^{-4}	0.86	Y= 6.7 + 1.6x
	72	0.052×10^{-4}	0.029×10^{-4} - 0.072×10^{-4}	0.34	Y= 7.3 + 1.8x
	84	0.041×10^{-4}	0.022×10^{-4} - 0.054×10^{-4}	0.82	Y=8.6 + 2.6x
T_6 : Flubendiamide (ml/lt)	48	1.4×10^{-4}	0.87×10^{-4} - 4.3×10^{-4}	0.26	Y=6.0 + 1.19x
	60	0.29×10^{-4}	0.084×10^{-4}- 0.46×10^{-4}	1.11	Y=6.98 + 1.29x
	72	0.14×10^{-4}	0.010×10^{-4} - 0.25×10^{-4}	2.22*	Y=8.07 + 1.67x
T_7 : Chlorantraniliprole (ml/lt)	48	0.17×10^{-4}	0.11×10^{-4} - 0.30×10^{-4}	1.20	Y=6.15 + 1.47x
	60	0.087×10^{-4}	0.061×10^{-4} –0.12×10^{-4}	2.22*	Y=6.98 + 1.87x
	72	0.054×10^{-4}	0.0033×10^{-4} –0.073×10^{-4}	2.87*	Y=7.74 + 2.16x
T_8 : Cyantraniliprole (ml/lt)	24	4.9×10^{-4}	3.4×10^{-4} –8.0×10^{-4}	0.23	Y=5.49 + 1.55x
	36	2.7×10^{-4}	1.9×10^{-4} –3.6×10^{-4}	1.23	Y=6.15 + 2.01x
	48	2.1×10^{-4}	1.4×10^{-4} –2.8×10^{-4}	4.94*	Y=6.55 + 2.25x

*: Significant at p=0.05. In treatment column parenthesis units mentioned of specific treatment.

Table 3: Median lethal time at recommended dose of different microbial and newer molecules against larvae of *H. armigera*

Treatment	LT_{50} (Hour)	Fiducial limit (Hour)	χ^2	Reg. Equation
T_1 *Beauveria bassiana*	154.63	144.41 – 164.83	4.72	Y= -10.74 + 7.19x
T_2 *Bacillus thuringiensis*	83.72	75.92 – 91.58	4.35	Y= -7.31 + 6.40x
T_3 HaNPV	114.58	105.87 – 123.13	3.87	Y= -10.10 + 7.33x
T_4 Emmamectin Benzoate	8.81	6.71 – 10.63	5.07	Y= 1.79 + 3.38x
T_5 Spinosad	44.62	40.31 – 48.89	4.66	Y= -4.42 + 5.71x

T_6 Flubendiamide	44.41	40.80 – 47.78	1.14	Y= -11.41+ 9.96x
T_7 Chlorantraniliprole	35.54	31.26 – 39.44	2.80	Y= -3.73 + 5.63x
T_8 Cyantraniliprole	15.09	5.99 – 37.96	9.37	Y= 0.16 + 4.10x

Fiducial limit calculated at p= 0.05

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