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Study the bio-efficacy of some biopesticide formulation against cabbage butterfly *Pieris brassicae* Linn. in mustard

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Abstract
An experiment was conducted at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand, to study the bioefficacy of some biopesticide formulation against cabbage butterfly *Pieris brassicae* Linn., In cabbage. Biopesticide used in the experiment were *Bacillus thuringiensis* (Lipel), *Verticillium lecanii* (Mealikil), *Beauveria bassiana* (Race), *Beauveria bassiana* (Daman), *Beauveria bassiana* (Local strain), *Metarhizium anisopliae* (Pacer), *Metarhizium anisopliae* (Kalichkra) and contol. The *Bacillus thuringiensis* (Lipel), *Verticillium lecanii* (Mealikil), *Beauveria bassiana* (Race), *Metarhizium anisopliae* (Pacer) and *Beauveria bassiana* (Local strain) were Ist 1x108, 2nd 5x107, 3rd 2x107 and 4th 1x107. The observations of mortality were recorded after 1st, 2nd, 3rd, 4th, 5th and 6th days of feeding at different concentrations. The final observation of bio-efficacy based on pooled mean values (mean of mortality values at all days and concentrations) was observed as *B. bassiana* (local strain) (49.9%)>Mealikil (47.2%)> Pacer (46.4%)>Kalichakra (45.8%)> Racer (45.7%)>Lipel (44.2%)> Daman (37.5%).

Keywords: *Pieris brassicae*, cabbage butterfly, Uttarakhand

Introduction
High incidence of cabbage butterfly, *P. brassicae* Linn. is one of the major constraints (Rai *et al.*, 1985, Mishra and Ram, 1997) [3, 2] in brassicas. The early instar larvae feed gregariously by scrapping the under surface of the leaves whereas the subsequent instars disperse as they grow up and eat up leaves from the margins inwards, leaving intact the main veins. Survey conducted from time to time in Uttarakhand revealed that intensive and continuous cropping and improper plant protection measures are the major factors contribute to the seriousness of *P. brassicae* (Singh and Tiwari, 2000) [4].

Materials and Methods
Pantnagar climate is somewhat humid, subtropical in nature, which is characterized by hot dry summer and cold winter. The temperature exceeds even 38°C in summer while in winter it may fall down to 4°C. The mean annual rainfall is approximately 140cm and the mean rainy season may spreads from the last week of June to middle of September.

Materials
i. Plastic jar
ii. Hand brush
iii. Forceps
iv. Conical flasks
v. Beakers
vi. Filter papers
vii. Muslin cloth
viii. Measuring cylinder
ix. Atomizer
x. Petri dishes
xi. Market formulation of following biopesticides were used (Table 1.1)

Table 1.

Biopesticide formulations		Concentrations in Colony Forming Unit (CFU)			
Microbial species as *a.i.*	Trade name	1st	2nd	3rd	4th
Bacillus thuringiensis	Lipel	1x108	5x107	2x107	1x107
Verticillium lecanii	Mealikil	1x108	5x107	2x107	1x107
Beauveriabassiana	Racer	1x108	5x107	2x107	1x107
Metarhiziumanisopliae	Pacer	1x108	5x107	2x107	1x107
Metarhiziumanisopliae	Kalichakra	2x108	1x108	5x107	2x107
Beauveriabassiana	Daman	1x109	5x108	2x108	1x108
Beauveriabassiana	Local strain	1x108	5x107	2x107	1x107

Maintenance of Insect Cultures

The cultures of different test insects were maintained in laboratory for the investigation. Gravid female of *L. erysimi*and 4th instar larvae of *P. brassicae*and *S. obliqua*were collected from CRC, Pantnagar and transferred to round glass jar covered with muslin cloths containing disinfected fresh mustard leaves as food. The larvae and aphids were transferred everyday into clear disinfected jars. The pupae of *P. brassicae*and *S. obliqua* were kept for adult emergence separately. The adults were transferred to oviposition jar lined with filter paper and cotton plug soaked in 15 percent sucrose solution were provided as food for adults. The paper pieces carrying egg masses were kept in another jar and were allowed to hatch, the neonate larvae were reared on mustard leaves to attain the age of second instar. In case of *L. erysimi*, one day old nymphs were removed from the jar and reared in mustard leaves at room temperature.

Determination of efficacy of some biopesticide formulations against thetestinsects by spray method

Bioassay

All the lab experiments were conducted in control room of laboratory at 27 ± 20C and 95 ± 5% relative humidity. The efficacy of biopesticide formulations was studied against test insects. Based on literature and the preliminary screening, concentrations of formulations were decided. The experiments were carried out in two phases, preliminary screening and final testing alternatively, to study the lethal concentration of formulations.

Application of biopesticides

For pathogenicity study, the 3d old nymphs of *L. erysimi* and second instar larvaeof *P. brassicae and S. obliqua* were taken from nucleus culture. Four concentration of biopesticide suspension were used for each test. Five nymphs and larvae were placed in petri plates containing fresh mustard leaves and sprayed directly with 2ml biopesticide suspension using hand atomizer. After air drying, the treated larvae were carefully transferred to individual sterile round plastic jars. Jars were covered with muslin cloth having provision for proper aeration. The larvae were maintained in an incubator at 27±10C and 95±5% RH.

Observation

The observations of mortality were recorded after 1st, 2nd, 3rd, 4th, 5th and 6th days of feeding at different concentrations.

Statistical analysis

The data thus obtained were analyzed through Two Factor Randomized Block Design (RBD) based on computer programme STPR2. LC50 values was determined using probit analysis (Finney, 1971) based computer programme STPR718 at the Computer Center, College of Basic Sciences and Humanities of this University.

Results and Discussion

The data on per cent mortality of *P. brassicae* caused by different biopesticide formulations has been presented in table 4.2 and figure 2. The perusal of the data revealed that on first day after application of biopesticide formulations, no significant difference was recorded in mortality of larvae due to different biopesticide formulations at their varying concentrations. However, 1x108 and 5x107 CFU concentrations of Mealikil and 1x108 CFU concentration of *B. bassiana*(local strain) caused highest mortality (13.33%). While no mortality was observed at 5x107, 2x107 and 1x107 CFU concentrations of Lipel and Racer; 1x108, 5x107 and 2x107 CFU concentrations of Kalichakra; 5x108, 2x108 and 1x108 CFU concentrations of Daman and 1x107 CFU concentration of Mealikil and Pacer.

On second day after application of biopesticide formulations, highest mortality (26.66%) were observed at 1x108, 2x108 and 1x109 CFU concentrations of Pacer, Kalichakra and Daman, respectively. However, no mortality was occurred at 5x107, 2x107 and 1x107 CFU concentrations of Lipel; 1x107 CFU concentration of Mealikil, Racer and Pacer; 5x107 CFU concentration of Kalichakra and 1x107 CFU concentration of *B. bassiana* (local strain) (Table 4.2)

On third day after application of biopesticide formulations, significant difference was recorded in mortality of larvae due to different biopesticide formulations at their varying concentrations. Highest mortality (46.66%) was observed at 2x108 and 1x109 CFU concentrations of Kalichakra and Daman, respectively followed by 1x108, 5x107 and 2x107 CFU concentrations of *B. bassiana* (local strain) and 1x108 CFU concentration of Pacer (40.00% mortality for each). Mortality (46.66%) at 2x108 and 1x109 CFU concentrations of Kalichakra and Daman were found significantly higher than at 5x107, 2x107 and 1x107 CFU concentrations of Lipel and Racer; 2x107 and 1x107 CFU concentrations of Mealikil; 1x107 CFU concentration of Pacer; 1x108, 5x107 and 2x107 CFU concentration of Kalichakra; 2x108 and 1x108 CFU concentrations of Daman and 1x107 CFU concentration of *B. bassiana* (local strain) While no mortality was observed at 1x107 CFU concentration of Racer (Table 4.2).

On fourth day after application of biopesticide formulations, significant difference was recorded in mortality of larvae due to different biopesticide formulations at their varying concentrations. Highest mortality (33.33%) were observed at 1x108 CFU concentrations of Pacer and *B. bassiana* (local strain) followed by 1x109 CFU concentration of Daman (66.66%); 2x108 CFU concentrationof Kalichakra (60.00%); 1x108 CFU concentrationof Mealikil (53.33%), Lipel (46.66%) and Racer (46.66%). Mortality at 1x108 CFU concentration of Pacer and *B. bassiana* (local strain) was found significantly higher than mortality at 5x107, 2x107 and 1x107 CFU concentrations of Lipel and Racer; 2x107 and

1x107 CFU concentrations of Mealikil and Pacer; 1x108, 5x107 and 2x107 CFU concentrations of Kalichakra; 5x108, 2x108 and 1x108 CFU concentration of Daman and 1x107 CFU concentration of *B. bassiana* (local strain) (Table 4.2).

On fifth day after application of biopesticide formulations, no significant difference was recorded in mortality of larvae due to different biopesticide formulations at their varying concentrations. However, highest mortality (80.00%) were observed at 1x108 CFU concentration of Pacer and *B. bassiana* (local strain) followed by 1x109 CFU concentration of Daman (66.66%); 2x108 CFU concentration of Kalichakra (60.00%); 1x108 CFU concentration of Racer (60.00%), Mealikil (60.00%) and Lipel (46.66%). Mortality recorded at highest concentrations was found significantly higher than the mortality at lowest concentrations except Mealikil. Lipel at 1x108 CFU concentration caused 46.66% mortality which was significantly lower than the mortality (80.00%) occurred at 1x108 CFU concentration of Pacer and *B. bassiana*(local strain). Mortality (46.66%) at 5x107 CFU concentration of Racer was found significantly higher than mortality (13.33%) at 2x107 CFU concentration of its own; mortality (80.00%) at 1x108 CFU concentration of Pacer was found significantly higher than at 2x107 and 1x107 CFU concentrations of its own *i.e.* 40.00% and 13.33% mortality, respectively. Mortality (53.33%) at 5x107 CFU concentration of Pacer was found significantly higher than mortality (13.33%) at 1x107 CFU concentration of its own and Mortality (66.66%) at 5x107 CFU concentration of *B. bassiana*(local strain) was found significantly higher than mortality (33.33%) at 1x107 CFU concentration of its own (Table 4.2).

On sixth day after application of biopesticide formulations, as in case of fifth day, no significant difference was recorded in mortality of larvae due to different biopesticide formulations at their varying concentrations. However, highest mortality (93.33%) was observed at 1x108 CFU concentrations of *B. bassiana* (local strain) followed by 1x108 CFU concentration of Mealikil (86.66%), Pacer (80.00%) and Racer (73.33%); 1x109 CFU concentration of Daman (66.66%); 2x108 CFU concentration of Kalichakra (60.00%) and 1x108 CFU concentration of Lipel (46.66%). Least mortality (13.33%) was recorded at 1x107 CFU concentration of Lipel and Racer, while no mortality was occurred at 1x107 CFU concentration of Racer (Table 4.2).

Mean percent mortality indicated that the highest mean mortality *i.e.* 53.4, 45.6, 37.8and 17.8% was observed in *B. bassiana* (local strain) at 1st, 2nd, 3rd and 4th concentrations, respectively. However, lowest mean mortality (29.9, 14.5, 8.9 and 6.7%) was observed in Lipel at 1st, 2nd, 3rd and 4th concentrations (Table 4.2). Thus, based on mean percent mortality, the order of bioefficacy of biopesticide formulations was observed as *B. bassiana* (local strain)> Pacer> Daman>Mealikil>Kalichakra>Racer>Lipel; *B. bassiana* (local strain)>Mealikil> Pacer> Racer> Daman>Kalichakra>Lipel; *B. bassiana* (local strain)> Pacer>Mealikil> Daman>Kalichakra> Racer>Lipel and *B. bassiana* (local strain)>Mealikil> Daman>Kalichakra> Pacer>Lipel> Racer at 1st, 2nd, 3rd and 4th concentrations, respectively. While the order of bioefficacy based on pooled mean values was observed as *B. bassiana* (local strain) (49.9%)>Mealikil (47.2%)> Pacer (46.4%)>Kalichakra (45.8%)> Racer (45.7%)>Lipel (44.2%)> Daman (37.5%) (Table 4.2). Similar work done by Zhang and his co-workers, (2001) [5] found that the pathogenicity of the *V. lecanii* (V-816 strain) to *P. brassicae* increased with increase of spore content. The spore content of 4 x 105/liter resulted in the mortality of 26.4% of 2-3 instar *P. brassicae*, but the mortality reached 70.8% when spore content increased to 4 x 108/liter. The pathogenicity of the V-816 strain to young larvae was greater than to older larvae.

The efficacy of *B. bassiana* (Bals.-Criv.) Vuill. native strain (BbPM) and *B. bassiana* (Bea-Sin™) and *M. anisopliae* (Metchnikoff) Sorokin (Meta-Sin™)-based commercial products were evaluated against the imported cabbageworm, *P. rapae* (L). Three concentrations (1.2×1012, 1.2×109, and 1.2×106 conidia per hectare) of BbPM, Bea-Sin™, and Meta-Sin™ were applied to commercial cabbage, *Brassica oleracea* var. *capitata* L., to evaluate larval mortality. Native strain BbPM (92.7%) and Bea-Sin™ (91.8%) killed significantly more larvae than did Meta-Sin™ (62.6%). (Campos *et al.*, 2010) [1].

In case of *P. brassicae*(Linn.), maximum mean percent mortality *i.e.* 53.4, 45.6, 37.8and 17.8% was observed by *B. bassiana* (local strain) at 1st, 2nd, 3rd and 4th concentrations, respectively. However, lowest mean mortality (29.9, 14.5, 8.9 and 6.7%) was observed by Lipel at 1st, 2nd, 3rd and 4th concentrations. Thus, the order of bioefficacy based on pooled mean values was observed as *B. bassiana* (local strain) (49.9%)>Mealikil (47.2%)> Pacer (46.4%)>Kalichakra (45.8%)> Racer (45.7%)>Lipel (44.2%)> Daman (37.5%).

Table 2: Bioefficacy of some biopesticide formulations against *Pieris brassicae* (Linnaeus)

	Days after application (DAA)																								Mean				Pooled
DAA	**1st**				**2nd**				**3rd**				**4th**				**5th**				**6th**				**Concentration**				**mean**
Conc./treat	**Concentration**				**Concentration**				**Concentration**				**Concentration**				**Concentration**				**Concentration**								
	1st	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	
Lipel (*Bacillus thuringiensis)*	6.6	0	0	0	6.6	0	0	0	20.2	6.6	6.6	6.6	46.6	13.3	13.3	6.6	46.6	33.3	13.3	13.3	53.3	33.3	20.0	13.3	29.9	14.5	8.8	6.6	14.96
Mealikil (*Verticillium lecanii*)	13.3	13.3	6.6	0	13.3	13.3	6.6	0	33.3	33.3	13.3	13.3	60.0	53.3	26.6	20.0	60.0	53.3	33.3	33.3	86.6	66.6	46.6	33.3	44.5	38.9	22.3	16.7	30.52
Racer (*Beauveriabassiana*)	6.6	0	0	0	6.6	6.6	6.6	0	26.6	13.3	13.3	0	46.6	40.0	13.3	0	60.0	46.6	13.3	0	73.3	60.0	26.6	0	36.7	27.8	12.3	0	19.13
Pacer (*Metarhiziumanisopliae)*	6.6	6.6	6.6	0	26.6	13.3	13.3	0	40.0	26.6	20.0	6.6	73.3	46.6	40.0	13.3	80.0	53.3	40.0	13.3	80.0	60.0	40.0	13.3	51.0	34.5	26.7	7.8	29.97
Kalichakra (*Metarhiziumanisopliae)*	6.6	0	0	0	26.6	6.6	0	6.6	46.6	6.6	6.6	6.6	60.0	13.3	20.0	13.3	60.0	33.3	33.3	13.3	60.0	46.6	33.3	20.0	43.4	17.8	15.6	9.9	21.63
Daman (*Beauveriabassiana*)	6.6	0	0	0	26.6	6.6	13.3	0	46.6	26.6	13.3	6.6	66.6	33.3	33.3	20.0	66.6	46.6	33.3	26.6	66.6	53.3	40.0	26.6	46.7	27.8	22.3	13.4	27.45
B. bassiana (Local strain)	13.3	6.6	6.6	6.6	20.0	20.0	13.3	6.6	40.0	40.0	40.0	6.6	73.3	60.0	46.6	13.3	80.0	66.6	53.3	33.3	93.3	80.0	66.6	40.0	53.4	45.6	37.8	17.8	38.57
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SEm+	4.23				5.13				6.21				7.44				8.26				8.51								
CD at 1%	NS				NS				23.37				27.98				NS				NS								
CD at 5%	NS				NS				17.57				21.04				NS				NS								

Concentrations: 1st = 1x108, 2nd = 5x107, 3rd = 2x107 and 4th = 1x107 for Lipel, Mealikil, Racer, Pacer and *Beauveriabassiana* (local strain), 1st= 2x108, 2nd = 1x108, 3rd = 5x107 and 4th= 2x107foe Kalichakar

1st= 1x109, 2nd= 5x108, 3rd= 2x108 and 4th = 1x108 foe Daman

Table 3: Bioefficacy of some biopesticide formulations against *Pieris brassicae* (Linnaeus)

	Days after application (DAA)																								Mean				Pooled
DAA	**1st**				**2nd**				**3rd**				**4th**				**5th**				**6th**				**Concentration**				**mean**
Conc./treat	**Concentration**				**Concentration**				**Concentration**				**Concentration**				**Concentration**				**Concentration**								
	1st	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**	
Lipel (*Bacillus thuringiensis)*	6.6	0	0	0	6.6	0	0	0	20.2	6.6	6.6	6.6	46.6	13.3	13.3	6.6	46.6	33.3	13.3	13.3	53.3	33.3	20.0	13.3	29.9	14.5	8.8	6.6	14.96
Mealikil (*Verticillium lecanii*)	13.3	13.3	6.6	0	13.3	13.3	6.6	0	33.3	33.3	13.3	13.3	60.0	53.3	26.6	20.0	60.0	53.3	33.3	33.3	86.6	66.6	46.6	33.3	44.5	38.9	22.3	16.7	30.52
Racer (*Beauveriabassiana*)	6.6	0	0	0	6.6	6.6	6.6	0	26.6	13.3	13.3	0	46.6	40.0	13.3	0	60.0	46.6	13.3	0	73.3	60.0	26.6	0	36.7	27.8	12.3	0	19.13
Pacer (*Metarhiziumanisopliae)*	6.6	6.6	6.6	0	26.6	13.3	13.3	0	40.0	26.6	20.0	6.6	73.3	46.6	40.0	13.3	80.0	53.3	40.0	13.3	80.0	60.0	40.0	13.3	51.0	34.5	26.7	7.8	29.97
Kalichakra (*Metarhiziumanisopliae)*	6.6	0	0	0	26.6	6.6	0	6.6	46.6	6.6	6.6	6.6	60.0	13.3	20.0	13.3	60.0	33.3	33.3	13.3	60.0	46.6	33.3	20.0	43.4	17.8	15.6	9.9	21.63
Daman (*Beauveriabassiana*)	6.6	0	0	0	26.6	6.6	13.3	0	46.6	26.6	13.3	6.6	66.6	33.3	33.3	20.0	66.6	46.6	33.3	26.6	66.6	53.3	40.0	26.6	46.7	27.8	22.3	13.4	27.45
B. bassiana (Local strain)	13.3	6.6	6.6	6.6	20.0	20.0	13.3	6.6	40.0	40.0	40.0	6.6	73.3	60.0	46.6	13.3	80.0	66.6	53.3	33.3	93.3	80.0	66.6	40.0	53.4	45.6	37.8	17.8	38.57
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SEm+	4.23				5.13				6.21				7.44				8.26				8.51								
CD at 1%	NS				NS				23.37				27.98				NS				NS								
CD at 5%	NS				NS				17.57				21.04				NS				NS								

Concentrations: 1st = 1x108, 2nd = 5x107, 3rd = 2x107 and 4th = 1x107 for Lipel, Mealikil, Racer, Pacer and *Beauveriabassiana* (local strain), 1st= 2x108, 2nd = 1x108, 3rd = 5x107 and 4th= 2x107foe Kalichakar
1st= 1x109, 2nd= 5x108, 3rd= 2x108 and 4th = 1x108 foe Daman

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