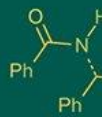


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In vitro bio-efficacy of selected insecticides against the cabbage butterfly, *Pieris brassicae* (L.) on cruciferous vegetable crops

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Abstract

The cabbage butterfly, *Pieris brassicae* (Lepidoptera: Pieridae), is an important pest of cruciferous vegetable crops and can cause substantial defoliation and yield losses. A laboratory study was conducted in 2023 at the Integrated Pest Management Laboratory, Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), to assess the bio-efficacy of selected insecticides and biopesticides against third-instar larvae of *P. brassicae*. The experiment was conducted in a completely randomized design with three replications using the leaf-dip method. Larval mortality was assessed at 24, 48, and 72 h after treatment, and mortality percentages were corrected using the appropriate control mortality. Among the treatments evaluated, chlorantraniliprole 18.5 EC at 0.3 ml L⁻¹ exhibited the highest efficacy, with 87.96% corrected mortality at 72 h, followed by spinosad 45 SC at 0.3 ml L⁻¹ (79.03%) and indoxacarb 15.8 EC at 0.5 ml L⁻¹ (77.99%). Emamectin benzoate showed intermediate efficacy, whereas *Metarhizium anisopliae*, *Verticillium lecanii*, and neem oil resulted in comparatively lower mortality. The findings demonstrate differential efficacy among the tested insecticides and biopesticides, with chlorantraniliprole showing the highest larvicidal activity against *P. brassicae* under laboratory conditions. These results provide useful information for the selection of effective insecticides for the management of *P. brassicae* in cruciferous vegetable crops.

Keywords: *Pieris brassicae*, bio-efficacy, insecticides, cruciferous, vegetables

1. Introduction

Cruciferous vegetables, including cabbage, cauliflower, broccoli, kale, knol khol and radish, are among the most important vegetable crops cultivated worldwide because of their high nutritional and economic value. India is one of the leading producers of cabbage and cauliflower, where these crops contribute significantly to vegetable production and farmers income. However, their productivity is greatly affected by several insect pests, among which the cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), is one of the most destructive species. The larvae of *P. brassicae* feed gregariously on leaves, causing severe defoliation, reduction in photosynthetic area and deterioration of market quality, ultimately resulting in considerable yield losses. The pest attacks cruciferous crops throughout their growth period and has been reported to cause yield losses ranging from 40-80% under severe infestation. Females lay eggs in clusters on the lower surface of leaves, and the emerging larvae feed collectively during the early instars before dispersing over the entire plant, resulting in extensive foliage damage. Management of *P. brassicae* has largely depended on the use of conventional insecticides. However, indiscriminate and repeated application of these chemicals has resulted in insecticide resistance, environmental contamination, destruction of beneficial organisms and pesticide residues in vegetables. Consequently, there is increasing interest in evaluating newer insecticides that provide effective control with lower environmental impact and greater selectivity toward target pests. Novel insecticides such as Chlorantraniliprole, Spinosad, Indoxacarb and Emamectin benzoate possess unique modes of action, high efficacy against lepidopteran pests and comparatively lower toxicity to non-target organisms. Evaluation of these insecticides under laboratory conditions is essential for identifying suitable molecules that can be incorporated into Integrated Pest Management (IPM) programmes.

Therefore, the present investigation was undertaken to evaluate the *in vitro* bio-efficacy of selected insecticides and bio-pesticides against third instar larvae of *Pieris brassicae* under laboratory conditions.

2. Materials and Methods

2.1 Experimental Site

The laboratory experiment entitled “In vitro Bio-efficacy of Selected Insecticides against the Cabbage Butterfly (*Pieris brassicae* L.) on Cruciferous Vegetable Crops” was conducted during the spring season of 2023 at the Integrated Pest Management (IPM) Laboratory, Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, Srinagar, India (34°14' N latitude, 74°88' E longitude; 1,592 m above mean sea level).

2.2 Maintenance of Laboratory Culture

A laboratory culture of *Pieris brassicae* was maintained using insecticide-free cauliflower plants. Eggs, larvae and pupae were collected from cruciferous vegetable fields and reared under controlled laboratory conditions to obtain healthy insects for bioassay studies.

2.3 Collection of Eggs

Egg masses of *P. brassicae* were collected from cabbage, cauliflower and kale fields (Fig 1). The eggs were placed in 9-cm Petri dishes lined with moist filter paper and maintained until hatching. Newly hatched larvae were immediately transferred to fresh cauliflower leaves for further rearing.

2.4 Rearing of Larvae

Larvae were reared in plastic containers (20 × 15 cm) and fed fresh insecticide-free cauliflower leaves daily (Fig 2). The rearing containers were cleaned regularly, and unhealthy or damaged larvae and pupae were discarded to maintain a healthy laboratory culture. Third instar larvae were used for bioassay experiments.

2.5 Rearing of Adults

Pupae were transferred to rearing cages (3 × 2 ft) for adult emergence. Adults were maintained on potted cauliflower plants and supplied with 10% sugar solution through cotton swabs, which were replaced daily. Fresh plants were provided regularly for oviposition.

2.6 Insecticides Evaluated

Seven treatments comprising four chemical insecticides, two entomopathogenic fungi, one botanical insecticide and an untreated control were evaluated against third-instar larvae of *P. brassicae*.

Treatment	Formulation	Dose
Chlorantraniliprole	18.5 EC	0.3 ml L ⁻¹
Spinosad	45 SC	0.3 ml L ⁻¹
Indoxacarb	15.8 EC	0.5 ml L ⁻¹
Emamectin benzoate	5 SG	0.4 ml L ⁻¹
Neem oil	1500 ppm	3 ml L ⁻¹
<i>Metarhizium anisopliae</i>	1.0 WP	5 ml L ⁻¹
<i>Verticillium lecanii</i>	1.0 WP	5 ml L ⁻¹
Control	Distilled water	—

2.7 Bioassay Procedure

The bio-efficacy of insecticides was evaluated using the leaf-dip method under laboratory conditions (25±1 °C, 65±5% relative humidity and 14:10 h light: dark photoperiod). Fresh cabbage leaves were dipped in the respective treatment solutions for 30 seconds, air-dried and placed individually in Petri dishes lined with moist filter paper. Thirty third instar larvae were released per treatment with three replications (90 larvae per treatment). Leaves treated with distilled water served as the untreated control. Larval mortality was recorded after 24, 48 and 72 hours.

2.8 Calculation of Larval Mortality

Larval mortality (%) was calculated using the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae released}} \times 100$$

2.9 Calculation of Corrected percentage mortality

Every 24 hours, 48 hours, and 72 hours, the corrected percentage mortality was calculated using the following formula.

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

T = % mortality in treated

C = % mortality in control

2.10 Statistical Analysis

The experiment was laid out in a Completely Randomised Design (CRD) with three replications. Corrected mortality data were subjected to analysis of variance (ANOVA), and treatment means were compared at the 5% level of significance.

3. Results and Discussion

3.1 *In vitro* Bio-efficacy of Selected Insecticides against *Pieris brassicae*

The bio-efficacy of seven insecticides and bio-pesticides against third instar larvae of *Pieris brassicae* was evaluated under laboratory conditions using the leaf-dip method. Corrected larval mortality (Fig 3) was recorded at 24, 48 and 72 hours after treatment (Table 1). Among all treatments, Chlorantraniliprole 18.5 EC @ 0.3 ml L⁻¹ was the most effective, recording the highest corrected larval mortality of 78.63%, 82.46% and 87.96% after 24, 48 and 72 hours, respectively. The superior performance of Chlorantraniliprole may be attributed to its novel mode of action on insect ryanodine receptors, resulting in rapid feeding cessation, paralysis and mortality of larvae (Cordova *et al.*, 2006) [2]. Spinosad 45 SC @ 0.3 ml L⁻¹ was the second most effective treatment, causing corrected mortalities of 70.43%, 73.44% and 79.03% after 24, 48 and 72 hours, respectively. Indoxacarb 15.8 EC @ 0.5 ml L⁻¹ also showed high efficacy with corrected mortalities of 71.53%, 75.66% and 77.99%, followed by Emamectin benzoate 5 SG @ 0.4 ml L⁻¹, which recorded 61.56%, 67.86% and 72.33% mortality during the corresponding observation periods. Among the bio-pesticides, Neem oil (1500 ppm) produced comparatively low larval mortality, ranging from 26.13% to 30.90%. Likewise, *Verticillium*

lecanii 1.0 WP and *Metarhizium anisopliae* 1.0 WP exhibited relatively poor efficacy, recording maximum corrected mortalities of 25.33% and 23.09%, respectively, at 72 hours after treatment. Larval mortality increased progressively from 24 to 72 hours after treatment in all treatments, indicating that prolonged exposure enhanced the insecticidal activity. Synthetic insecticides exhibited faster and higher larvicidal activity than the bio-pesticides, whereas the latter showed comparatively slower action due to their biological mode of action. The comparatively lower efficacy of Neem oil, *Verticillium lecanii* and *Metarhizium anisopliae* may be attributed to their slower mode of action. Unlike synthetic insecticides, these bio-pesticides require time to infect or disrupt the physiological processes of the insect, resulting in delayed larval mortality. Overall, Chlorantraniliprole was the most effective treatment, while Spinosad and Indoxacarb exhibited comparable efficacy, followed by Emamectin benzoate, Neem oil, *Verticillium lecanii* and *Metarhizium anisopliae*.

The superior efficacy of Chlorantraniliprole observed in the present study is consistent with previous reports demonstrating its high activity against lepidopteran larvae. The comparatively higher efficacy of Spinosad and Indoxacarb also reflects their rapid neurotoxic action against lepidopteran larvae, whereas the lower efficacy of the bio-pesticides may be associated with their slower infection process. Likewise, Dhawan *et al.* (2010) [3] reported greater toxicity of Chlorantraniliprole and Spinosad compared with conventional insecticides against *P. brassicae*. These findings collectively support the superior efficacy of newer insecticides, particularly Chlorantraniliprole and Spinosad,

against lepidopteran pests. Minor variations in larval mortality reported among different studies may be attributed to differences in larval stage, insecticide concentration, environmental conditions and experimental methodology. The present findings are in agreement with Abbas *et al.* (2015) [1], who reported the superior efficacy of Chlorantraniliprole against lepidopteran larvae. Similar observations were reported by Su *et al.* (2017) [4], who found Chlorantraniliprole, Spinosad and Indoxacarb to be highly effective against *Pieris rapae* on cabbage. The sustained increase in larval mortality from 24 to 72 hours indicates the good residual activity of Chlorantraniliprole, Spinosad and Indoxacarb. Their prolonged effectiveness against *P. brassicae* larvae may contribute to improved pest suppression and reduced frequency of insecticide applications.

The observed effectiveness of Chlorantraniliprole, Spinosad and Indoxacarb confirms their potential for the management of *P. brassicae* under laboratory conditions. Owing to their superior performance, Chlorantraniliprole, Spinosad and Indoxacarb may be considered promising components of integrated pest management (IPM) programmes. However, these insecticides should be used judiciously and rotated with bio-pesticides and insecticides having different modes of action to minimise the risk of resistance development. However, further field evaluation under different agro-climatic conditions is necessary to validate their performance before recommending their large-scale use. The present findings provide useful information for selecting effective insecticides for the sustainable management of *P. brassicae* in cruciferous vegetable crops.

Table 1: Percent mortality of 3rd instar larvae of *P. brassicae* with different chemical insecticides and bio-pesticides

S. No	Treatment	Active ingredients	Dose	Cumulative Corrected mortality		
				24hrs	48hrs	72hrs
1	Chlorantraniliprole	18.5% EC	0.3 ml/lit	78.63 (62.44)	82.46 (65.22)	87.96 (69.67)
2	Spinosad	45 SC	0.3 ml/lit	70.43 (57.03)	73.44 (58.95)	79.03 (62.72)
3	Indoxacarb	15.80 EC	0.5 ml/lit	71.53 (57.73)	75.66 (60.41)	77.99 (61.93)
4	Emamectin Benzoate	5 SG	0.4 ml/lit	61.56 (51.66)	67.86 (55.44)	72.33 (58.24)
5	Neem Oil	1500 ppm	3 ml/lit	26.13 (30.73)	29.83 (33.09)	30.9 (33.75)
6	<i>Metarhizium anisopliae</i>	1.0 WP	5 ml/lit	19.5 (26.19)	21.2 (27.40)	23.09 (28.71)
7	<i>Verticillium lecanii</i>	1.0 WP	5 ml/lit	22.06 (28.00)	23.43 (28.94)	25.33 (30.20)
8	Control (water)	-	-	0 (1.00)	0 (1.00)	0 (1.00)
Se(d)				0.805	0.504	0.666
C.D.				1.611	1.009	1.333

4. Conclusion

The present laboratory investigation demonstrated significant differences in the efficacy of the tested insecticides against third instar larvae of *Pieris brassicae*. Among all treatments, Chlorantraniliprole 18.5 EC @ 0.3 ml L⁻¹ was the most effective, recording the highest corrected larval mortality (87.96%) after 72 hours, followed by Spinosad 45 SC @ 0.3 ml L⁻¹ (79.03%) and Indoxacarb 15.8 EC @ 0.5 ml L⁻¹ (77.99%). Emamectin benzoate showed moderate efficacy, whereas Neem oil, *Verticillium lecanii*

and *Metarhizium anisopliae* exhibited comparatively lower effectiveness under laboratory conditions. The findings indicate that Chlorantraniliprole, Spinosad and Indoxacarb are promising insecticides for the effective management of *P. brassicae* larvae in cruciferous vegetable crops. However, field evaluation under different agro-climatic conditions is necessary to validate their performance and determine their suitability for incorporation into integrated pest management (IPM) programmes.



Fig 1: Eggs of *P. brassicae*



Fig 2: Rearing of *P. brassicae* on cabbage leaves



Fig 3: Dead larvae of *P. brassicae*

accordance with appropriate scientific and ethical standards. All authors contributed to the study, reviewed and approved the final version of the manuscript, and agree to be accountable for all aspects of the work. The authors declare that the manuscript is original and has not been published previously, nor is it currently under consideration for publication elsewhere.

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Credit to Authorship

Saleema Jan: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, and Writing-original draft. Irfan Manzoor: Methodology, Investigation, Data collection, and Formal analysis. Owais B. Itoo: Investigation, Data collection, Data curation, and Validation. Shaheen Gul: Methodology, Investigation, Validation, and Writing-review and editing. Malik Mukhtair: Investigation, Data curation, Formal analysis, and Writing-review and editing. Aabid A. Bhat: Writing-review and editing.

Authors' Declaration Statement

The authors declare that there are no conflicts of interest associated with this work. The study was conducted in