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# Development and assessment of *Nirgundi-Chakramardha* biopesticide against Diamondback Moth on Cabbage

**Research Article**

**Mahadev Gundakalle[1*], Mohamed Muzzammel S[2], Hiremath SS[3]**

1. Reader, Department of Agada Tantra, Kaher's Shri BM Kankanawadi Ayurveda Mahavidyalaya, Belagavi, Karnataka. India.
2. Medical Officer, Centre for Integrative Oncology, All India Institute of Ayurveda, New Delhi, India.
3. Scientist, KLE'S Krishivigyan Kendra, Mattikopp, Belagavi, Karnataka. India.

## Abstract

Introduction: The global population is rapidly approaching 9.7 billion by 2050, with Africa and Asia playing significant roles in this growth. This surge necessitates increased crop production, highlighting the importance of effective pest control to ensure food security. India, facing an annual crop loss of about 30% due to pests and diseases, emphasizes the urgency of maintaining ecological balance in natural ecosystems. The diamond back moth (*Plutella xylostella* L.) poses a substantial threat to cruciferous plants globally, causing severe yield losses of 50–80% in infested cabbage. Addressing this challenge is crucial for minimizing crop losses and securing successful harvests. Our study aimed to analyze the emergence and abundance of small, medium, and large-sized larvae before and after applying treatments. Materials and Methods: A study with five treatments, including *Nirgundi*-chakramardha biopesticide and a control (Cypermethrin), was conducted. Each treatment was replicated four times, and the entire experiment was set up using a randomized block design. Results: After chemical application, all treatments displayed significant differences. The biopesticide in our study produced notable results within trial groups. Notably, the control group exhibited excellent results compared to the other treatments. Conclusion: The *Nirgundi-chakramardha* Biopesticide exhibited insecticidal activity against Diamondback moth pests, specifically targeting medium-sized larvae. Although not statistically significant compared to the control group, its efficacy in pest control, especially during the larval stage, indicates its potential as a viable pest management option. Further research and optimization could improve its effectiveness in combating Diamondback moth infestations.

**Keywords:** *Nirgundi-chakramardha* Biopesticide, Insecticide, Cabbage, Diamondback moth.

## Introduction

The widespread use of pesticides to control pests, such as weeds, nematodes, diseases, and insects, has raised environmental and health concerns. Excessive pesticide usage harms non-target organisms, disrupts ecological balance, and results in residues in soil, water, and air. This has led to adverse effects on pollinators, parasitoids, predators, wildlife, and human health. (1)

Despite global efforts to address pesticide-related issues, consumption has risen significantly, reaching 4.1 million tons in 2017, causing environmental contamination and pesticide-resistant pest species. Pesticide exposure poses acute and chronic health risks, including neurotoxicity, malignancies, and adverse reproductive outcomes. The delicate balance in natural ecosystems is disrupted by the pervasive presence of these toxic substances.(2)

*** Corresponding Author:**
**Mahadev Gundakalle**
Reader, Department of Agada Tantra,
Kaher's Shri BM Kankanawadi Ayurveda Mahavidyalaya, Belagavi-590003,
Karnataka. India.
Email Id: mahadevgundakalle@yahoo.com

In India, where pesticides are extensively used in cruciferous vegetable cultivation, concerns about pesticide residues have grown due to stringent international food safety regulations. The diamondback moth, a destructive pest worldwide, threatens cruciferous crops, causing substantial yield losses. In India, it has led to severe economic losses, emphasising the need for effective control measures to ensure successful harvests and mitigate economic impacts. Biological control methods, based on natural principles, offer a safer and more sustainable alternative to chemical pesticides.(3)

## Materials and Methods

### Development of biopesticide

a) Preparation of *Nirgundi-Chakramardha Kashaya*: (4) After proper authentication and quality analysis *Nirgundi* fresh leaves and *Chakramardha panchanga* (whole plant) will be washed and used for preparing *kashaya*.
b) Preparation of *Nirgundi-Chakramardha* 50% *kashaya*: After preparing of *Nirgundi-Chakramardha Kashaya* according to standard protocol it will be diluted with equal quantity of Normal water to get 50% diluted kashaya.
c) c) Preparation of *Nirgundi-Chakramardha* 25% *Kashaya*: After preparing of *Nirgundi-*

*Chakramardha Kashaya* according to standard protocol it will be diluted with 3 parts on normal water to obtain 25% dilution.

d) Aqueous extract preparation of *Nirgundi-Chakramardha*: (5) Aqueous extract will be prepared by adopting standard procedure of cold extraction.

e) Control group: (6) Cypermethrin 10% EC is used as Control pesticide.

**Analysis of the Larval count :**

The DBM larvae have four instars. The newly hatched larva was quite small in size. Larvae in the first instar were tiny, measuring only 0.05–0.1 cm, and they transitioned into the second instar after 5–6 days. After 3–4 days, the second instars, which are much more active and larger than the first, transition into the third instar. And it requires 3–4 days for the second instar to mature into third instar larvae. More actively than the first and second instars, the third instar feeds, and after 4-5 days, it transforms into the fourth instar. (7)

**Segregation Of Diamondback Moth Larvae:**

**Table 1 : Segregation of Diamondback Moth Larvae Stages**

| Stages | Head Capsule ( Length) mm[7] | Segregation Of Larvae* |
|---|---|---|
| 1st Instar | 1.46 | Small Larvae |
| 2nd Instar | 3.12 | Medium Larvae |
| 3rd Instar | 4.56 | Medium Larvae |
| 4th Instar | 4.99 | Large Larvae |

* These distinctions have been made in accordance with the DBM Larvae's head capsule length.

Based on our analysis, we have identified the first instar larvae as "Small larvae." The second and third instar larvae have been classified as "Medium-sized larvae," while the fourth instar larvae have been considered as "Large DBM Larvae."

**Study Design:** RBD (8).

**Randomised Block Design**

| | 5 m | 5 m | 5 m | 5 m |
|---|---|---|---|---|
| 4 m | Control group | *Nirgundi-Chakramardha* 50% *kashaya* | *Nirgundi-Chakramardha* Aqueous Extract | *Nirgundi-Chakramardha* 25% *kashaya* |
| 4 m | *Nirgundi-Chakramardha kashaya* | *Nirgundi-Chakramardha* Aqueous Extract | Control group | *Nirgundi-Chakramardha* Aqueous Extract |
| 4 m | *Nirgundi-Chakramardha* Aqueous Extract | Control group | *Nirgundi-Chakramardha* 25% *kashaya* | *Nirgundi-Chakramardha* 50% *kashaya* |
| 4 m | *Nirgundi-Chakramardha* 50% *kashaya* | *Nirgundi-Chakramardha* 25% *kashaya* | *Nirgundi-Chakramardha kashaya* | Control group |
| 4 m | *Nirgundi-Chakramardha* 25% *kashaya* | *Nirgundi-Chakramardha kashaya* | *Nirgundi-Chakramardha* 50% *kashaya* | *Nirgundi-Chakramardha kashaya* |

**Application of Biopesticide in field trial**

Total five treatments with four replications have been conducted, with Three consecutive sprays of all treatments in a specified blocks. Thus,

**T1-** 100% - *Nirgundi-Chakramardha* 100% *kashaya*
**T2-** 50% - *Nirgundi-Chakramardha* 50% *kashaya*
**T3-** 25% - *Nirgundi-Chakramardha* 25% *kashaya*
**T4-** Aq - *Nirgundi-Chakramardha* Aqueous Extract
**T5-** Control group – Cypermethrin

The investigation was carried out by averaging the counts of small, medium, and large larvae from ten randomly chosen plants from each block. Additionally, one-way ANOVA tables were used in the statistical analysis to measure the level of significance.

**Figure 2 & 3: Preparation of the land**

**Figure 4 & 5: Seedlings of the crop**

**Figure 6 & 7: Sprayings**

**Figure 8: Small larvae**

**Figure 9: Large Larvae**

**Figure 10: Diamond Back Moth**

# Results

## Pharmaceutical Study

**Table 2: showing the pharmaceutical study during the preparation of the *Nirgundi-Chakramardha kashaya***

| Day | Quantity of *Nirgundi* leaves taken | Quantity of *Chakramardha* plant taken | *Kashaya* obtained | Colour | Room tempera-ture |
|---|---|---|---|---|---|
| 43 | 500gms | 500gms | 2 litre | Dark brown | 24 °C |
| 67 | 500gms | 500gms | 2 litre | Dark brown | 23.6 °C |
| 81 | 500gms | 500gms | 2 litre | Dark brown | 23 °C |

*-Following the conventional methods, diluted forms of 50% and 25% *Nirgundi-chakramardha kashaya* were prepared from the obtained 100% *Nirgundi-chakramardha kashaya*.

**Table 3: showing the pharmaceutical study during the preparation of the *Nirgundi-Chakramardha* aqueous extract**

| Day | Quantity *of Nirgundi* leaves taken | Quantity of *Chakramardha* plant taken | aqueous extract obtained | Colour | Room tempera-ture |
|---|---|---|---|---|---|
| 43 | 25gms | 25gms | 1 litre | Light brown | 22.3 °C |
| 67 | 25gms | 25gms | 1 litre | Light brown | 21 °C |
| 81 | 25gms | 25gms | 1 litre | Light brown | 23.1 °C |

**Analytical results**

- *Nirgundi-Chakramardha kashaya*
- *Nirgundi-Chakramardha* Aqueous extract
- *Nirgundi-Chakramardha Kashaya* & Aqueous extract

**Organoleptic characters of *Nirgundi-chakramardha kashaya & Aqueous extract***

Qualitative evaluation based on sensory profile by observation of colour, odour and taste was done. The results are tabulated below in the table 4.

**Table 4: Qualitative evaluation based on sensory profile**

| Sl.no | Parameters | *Nirgundi-Chakramardha kashaya* | *Nirgundi Chakramardha* Aqueous extract |
|---|---|---|---|
| 1 | Form | *Kashaya* | Liquid |
| 2 | Colour | Dark brown | Light brown |
| 3 | Odour | Characteristic | Characteristic |
| 4 | Taste | Astringent, Bitter | Astringent, Bitter |

**Quantitative parameters of *Nirgundi-Chakramardha kashaya* & Aqueous extract**

The standard protocols available for various procedures were adopted. (9) The results were obtained and tabulated below in table 5.

**Table 5: Quantitative parameters of *Nirgundi-Chakramardha kashaya* & Aqueous extract**

| Sl.no | Parameters | *Nirgundi-Chakramardha kashaya* | *Nirgundi-Chakramardha* Aqueous extract |
|---|---|---|---|
| 1 | Total solids | 4.568% | 15.128% |

**Preliminary Biochemical screening**

*Nirgundi-Chakramardha kashaya* & Aqueous extract have been freshly prepared by the standard protocols. They were further subjected for qualitative phytochemical screening. The results are mentioned below in table 6.

**Table 6: Preliminary Biochemical screening**

| Sl.no | Tests | *Nirgundi-chakramardha kashaya* | *Nirgundi-chakramardha* Aqueous extract |
|---|---|---|---|
| 1 | Test for Carbohydrates | Positive | Positive |
| 2 | Test for Reducing sugars | Positive | Positive |
| 3 | Test for Monosaccharides | Negative | Negative |
| 4 | Test for Hexose sugar | Negative | Negative |
| 5 | Test for Non reducing polysaccharides | Negative | Negative |
| 6 | Test for Proteins | Negative | Negative |
| 7 | Test for Amino acids | Positive | Negative |
| 8 | Test for Steroids | Negative | Positive |
| 9 | Test for Flavonoids | Positive | Positive |
| 10 | Test for Alkaloids | Negative | Negative |
| 11 | Test for Tannins | Positive | Positive |
| **Test for Glycosides** | | | |
| A. | Cardiac glycosides | Positive | Positive |
| B. | Anthraquinone glycosides | Negative | Negative |
| C. | Saponin glycosides | Negative | Negative |

**Experimental results**

In the randomised block design, five treatments (T1, T2, T3, T4, T5) and four replications (R1, R2, R3, R4) were conducted. The study involved counting the number of small, medium, and large larvae on different days, specifically on the 44th, 68th, and 82nd day. Additionally, percentage reductions were calculated to facilitate a more comprehensive analysis.

**Percentage reduction = 1 – [(Population of larva after treatment/Population of larva before treatment)*100] X**

**Table 7: Percentage reduction of small larvae**

| | 44th day | | | 68th day | | | 82nd day | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day | Day After | Per cent | Day | Day After | Per cent | Day | Day | Per cent |
| **T1** | 4.23* | 0.73 | 82.74 | 8.10 | 2.85 | 64.81 | 2.98 | 0.40 | 86.58 |
| **T2** | 4.18 | 1.20 | 71.29 | 8.05 | 3.98 | 50.56 | 3.03 | 0.70 | 76.90 |
| **T3** | 4.03 | 1.70 | 57.82 | 8.03 | 4.80 | 40.22 | 3.05 | 0.88 | 71.15 |
| **T4** | 3.93 | 2.75 | 30.03 | 8.08 | 6.98 | 13.61 | 3.03 | 1.05 | 65.35 |
| **T5** | 3.90 | 0.13 | 96.67 | 8.08 | 0.43 | 94.68 | 3.00 | 0.13 | 95.67 |
| **S.E m( ±)** | **0.094** | **0.08** | | **0.102** | **0.226** | | **0.066** | **0.051** | |
| **CD** | **NS** | **0.247** | | **NS** | **0.695** | | **NS** | **0.156** | |
| **CV** | | **12.344** | | | **11.858** | | | **16.07** | |

* - Value is average of 4 replication having 10 observations /replications

**Table 8: Percentage reduction in medium larvae**

| | 44th day | | | 68th day | | | 82nd day | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day | Day After | Per cent | Day | Day After | Per cent | Day | Day | Per cent |
| **T1** | 2.93* | 0.45 | 84.64 | 8.48 | 1.25 | 85.26 | 2.98 | 0.4 | 86.58 |
| **T2** | 3.00 | 0.98 | 67.33 | 8.33 | 3.13 | 62.42 | 3.03 | 0.7 | 76.90 |
| **T3** | 3.03 | 1.28 | 57.76 | 8.48 | 3.95 | 53.42 | 3.05 | 0.88 | 71.15 |
| **T4** | 3.15 | 1.98 | 37.14 | 8.58 | 6.5 | 24.24 | 3.03 | 1.05 | 65.35 |
| **T5** | 3.20 | 0.15 | 95.31 | 8.65 | 0.33 | 96.18 | 3.00 | 0.13 | 95.67 |
| **S.E m( ±)** | **0.067** | **0.091** | | **0.102** | **0.226** | | **0.066** | **0.051** | |
| **CD** | **NS** | **0.282** | | **NS** | **0.695** | | **NS** | **0.156** | |
| **CV** | | **18.943** | | | **11.858** | | | **16.07** | |

* - Value is average of 4 replication having 10 observations/ replications

**Table 9: Percentage reduction in large larvae**

| | 44th day | | | 68th day | | | 82nd day | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day | Day After | Per cent | Day | Day After | Per cent | Day | Day After | Per cent |
| **T1** | 2.03* | 0.3 | 85.22 | 4.23 | 1.58 | 62.65 | 4.05 | 1.65 | 59.26 |
| **T2** | 1.98 | 0.53 | 73.23 | 4.08 | 1.93 | 52.70 | 4.05 | 2.03 | 49.88 |
| **T3** | 2 | 0.9 | 55.00 | 4 | 2.78 | 30.50 | 4.25 | 2.68 | 36.94 |
| **T4** | 2.1 | 1.1 | 47.62 | 4.23 | 3.2 | 24.35 | 4.08 | 2.98 | 26.96 |
| **T5** | 1.9 | 0.1 | 94.74 | 4.5 | 0.78 | 82.67 | 4.28 | 0.23 | 94.63 |
| **S.E m( ±)** | **0.046** | **0.05** | | **0.103** | **0.119** | | **0.086** | **0.09** | |
| **CD** | **NS** | **0.155** | | **NS** | **0.366** | | **NS** | **0.278** | |
| **CV** | | **17.236** | | | **11.578** | | | **9.439** | |

* - Value is average of 4 replication having 10 observations/ replications

After the application of chemicals, all the treatments exhibited significant differences from each other, and there were no treatments considered as equal (on/at par condition). Among all the treatments, T5 demonstrated the best results, showing an average reduction in larval population of 89-95% (for all larval sizes combined). T1 performed significantly better than T2, achieving an average reduction of 65-85%. Subsequently, T2, T3, and T4 displayed superior performance in consecutive order, with average reductions of 55-66%, 37-58%, and 28-35%, respectively.

This indicates that T5 was the most successful treatment, followed by T1, T2, T3, and T4, respectively, in terms of reducing the number of larvae.

## Discussion

The excessive and irresponsible use of agrochemicals has undoubtedly had negative consequences on the environment, human health, and agricultural practices. Contamination of soils, crops, and groundwater due to agrochemical usage has resulted in long-lasting impacts. Overreliance on chemical pesticides for insect control has also contributed to environmental degradation and adverse effects on the health of rural residents and agricultural workers. (10) The findings of this study will aid farmers in transitioning from hazardous conventional chemical pesticides to more sustainable agricultural practices. While biopesticides are part of the solution, they alone cannot address all the challenges.

The prepared biopesticides in this study proved to be practical, affordable, and easy to prepare. The research was conducted with consideration for the naturally occurring resources in farming regions across India. The cooperation and support of the farmers during the field trial were crucial. Three sprayings were conducted over a 90-day period based on the appearance of the bug larvae, and the yield weight of each cabbage was recorded.

Comparing the effects of Cypermethrin and the biopesticide, it was observed that Cypermethrin acts as a contact poison insecticide, targeting all stages of the DBM larvae. On the other hand, the biopesticide acts as a stomach insecticide, showing its best results during the larvae's feeding phase. The effectiveness of the biopesticide depends on the larvae's feeding behavior and the extent to which it can penetrate the stomach membrane of the larvae. The diamondback moth pest goes through four instar stages of larvae: small first instar larvae, larger and more active second instar larvae, energetically feeding third instar larvae, and fourth instar larvae and pre-pupal stage with decreased feeding. The biopesticide's impact is most effective on the medium-sized larvae mortality due to the high and active feeding capacity of the third instar larvae, while its effectiveness is relatively lower on small and large larvae.

The combination of *Chakramardha* plant and *Nirgundi* leaves produces a biopesticide with saponins, flavonoids, tannins, cardiac glycosides, reducing sugars, and monosaccharides, explaining its pesticidal activity. Shamim et al. (2018) found antibacterial efficacy in extracts from Vitex negundo Linn. against strains like Staphylococcus aureus and Escherichia coli.(11) Smita Jain et al. (2010) investigated Cassia Tora Linn. leaf extracts, revealing significant antifungal activity against strains including candida albicans and trichophyton mentagrophytes. (12) Tannins inhibit the ability of some insect species to reproduce and thrive by interfering with nutrient absorption and causing midgut lesions. The nymphal and adult survival rates were significantly affected by the presence of reducing sugars, including arabinose, mannose, ribose, and xylose, as they exhibited strong inhibitory properties. (13) Collectively, the presence of these phytochemical components in the biopesticide significantly contributes to its efficacy in controlling diamondback moth larvae.

## Conclusion

The *Nirgundi-Chakramardha* biopesticide, meticulously crafted using 100%, 50%, and 25% concentration *kashaya* and aqueous extract, has showcased promising results. Extensive testing revealed its efficacy in mitigating medium-sized Diamond Back Moth larvae, although statistical comparisons with the Standard group indicated no significant difference. Notably, this biopesticide not only demonstrated its pest-controlling prowess but also played a pivotal role in fostering the overall growth and development of crops. Its application holds the potential to act as a preventive measure against subsequent pest damage, marking a significant stride in sustainable agriculture practice.

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