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Antilarvicidal Potential of *Andrographis paniculata* Extracts Against *Plutella xylostella*

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Abstract

The plant extracts are beneficial to both human health and the environment. This research explores the impact of plant extracts against anti larvicidal potential of Diamond Back Moth (DBM). This study focuses on the collecting, rearing, and observation of the DBM (Plutella xylostella), with a particular focus on the influence of Andrographis paniculata (Nilavembu) plant extract on DBM pupal-adult metamorphosis. This study entailed collecting adult DBMs and their larvae from cauliflower plants in the Yercaud hills, followed by laboratory rearing under controlled conditions. The soxhlet extraction technique was used to produce a crude plant extract from A. paniculata. This plant extract was applied topically to newly hatched larvae, while control larvae were given a carrier solvent (acetone) treatment. The research purpose is to assess whether the normal pupal-to-adult transition of DBM was hindered by the application of A. paniculata plant extract. The emerging adults treated with the plant extract showed substantial variations in morphology and larval mortality when compared to the control group. In addition, the DBM's life cycle, including the stages of eggs, larvae, pupae, and adults, was thoroughly investigated. The study sheds light on the adverse effects of A.paniculata aqueous extract on the development of P. xylostella and proposes potential uses for pest control tactics.

Keywords: *Larval Mortality, Morphological Differences, Plant Extract, Plutella xylostella.*

Introduction

Any substance or combination of substances meant to prevent, eradicate, repel, or mitigate any pest can be defined as a pesticide [1]. Insects and pests have a continuous impact on global food production during crop growth, harvest, and storage. The annual crop production worldwide is estimated to have decreased by 18–20% [2]. Chemical pesticides can cause long-term ecological imbalances as well as health risks for both people and wildlife because they can remain in the environment and contaminate soil, water, and air [3]. Green pesticides, derived from natural sources like botanical extracts, microorganisms, or insect pheromones, provide an eco-friendly alternative to traditional chemical pesticides. With a shorter residual effect, they reduce pollution to the environment and harm to non-target organisms, such as wildlife and beneficial bugs [4]. The shift to green pesticides is motivated by increasing awareness of the

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detrimental impacts of chemical pesticides on ecosystems, human health, and non-target organisms. In contrast, green pesticides focus on effective pest control while minimizing harm to the environment and human health, promoting a sustainable and balanced agricultural approach [5].

Diamondback moth (DBM), scientifically known as *P. xylostella*, is a highly destructive pest found globally and is particularly harmful to cruciferous crops [6]. It has polyphagous larvae which cause damage to buds, leaves, seed buds and flowers of harvested cruciferous plants [7]. Also in tropical regions, *P. xylostella* may cause a lot of damage throughout the year, sometimes leading to staggering crop losses of up to 90% during larval outbreaks in Southeast Asia [8]. DBM is notoriously challenging to control due to several factors, including the absence of natural enemies like parasitoids and predators in many non-native regions, its migrant capabilities, and its elevated reproductive rate [9]. To control DBM, we can use plant-based products like crude extracts, botanical insecticide formulations and active chemical compounds [10]. *A. paniculata*, generally referred to as the "King of Bitters," is a therapeutic medicinal plant renowned for its potent medicinal properties. It is a key herb in traditional medicine systems, particularly in Ayurveda and traditional Chinese medicine, where it has been used for centuries to treat various ailments [11]. One of the lesser-known but significant attributes of *A.paniculata* is its anti-larvicidal activity against pests like the diamondback moth [12]. Studies have indicated that extracts from *A.paniculata* possess larvicidal properties, effectively inhibiting the growth and development of diamondback moth larvae. This natural insecticidal property makes *A.paniculata* an environmentally friendly and sustainable option for controlling agricultural pests while minimizing the use of harmful synthetic pesticides [13]. In this study, *A.paniculata* demonstrates promising anti-larvicidal activity against *P. xylostella*, offering a sustainable and eco-friendly alternative to chemical pesticides for pest control in agriculture.

Materials and Methods

Insect Collection and Rearing

Diamondback moths (DBM) in their adult and larval stages were collected from cauliflower plants in the Yercaud hills (Figure 1). The moths were reared in a laboratory at a regulated temperature of 28±2°C and humid condition of 65±2% to observe their life cycle. Food materials were provided in trays covered with fine wire mesh [14].

Figure 1. Diamondback Moth (*Plutella xylostella)*

Taxonomy of Insect

Name: Diamondback Moth
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Plutellidae
Genus: *Plutella*
Species: *xylostella*

Insect Culture

The DBM was collected from Yercaud Hills and placed in a container for larval culture. The larvae were identified and reared at 25°C and 70% relative humidity [15]. The first instar larvae were transferred to screen cages (42 × 43 × 55 cm) in laboratory at a temperature of 25±5°C. Captured larvae and pupae were kept apart in cylindrical plastic boxes measuring 3 cm × 7 cm, which contain lids to allow airflow. After being fed the leaves of host plants (cauliflower, cabbage), the larvae were monitored until the moth developed [16]. After developing, adult DBM were collected and placed in a cubic cage with a side length of 500 mm. On cauliflower plants, eggs were collected every day to aid in the development of larvae. Subsequently, the larvae were placed in a large plastic box measuring 28 cm × 27 cm, along with fresh leaves. A controlled climate room with a temperature of 25°C was used for DBM rearing.

Collection of Plant Material

A. paniculata leaves were collected from Salem (Figure 2). Healthy plant materials were brought to the laboratory, washed, and shade-dried for 2 weeks.

Taxonomy of Plant

Figure 2. Leaves of *Andrographis paniculata*

Botanical Name: Andrographis paniculata
Kingdom: Plantae
Order: Lamiales
Family: Acanthaceae
Genus: Andrographis
Species: paniculata

Extract Preparation

The crude plant extract was obtained through the soxhlet extraction technique. Around 20 grams of powdered plant material were placed in a thimble and extracted using 250 ml of methanol. The extraction process lasted for 24 hours or until the solvent in the siphon tube lost its colour [17]. Following

extraction, the solvent was evaporated on a hot plate maintained at 30°- 40°C until completely dry. The resulting dried extract was then stored in a refrigerator at 4°C for later use.

Application of Extract on Larvae

Freshly hatched larvae were topically treated on the abdominal region with 1 µ/µL of plant extract using acetone as the carrier solvent. Thirty larvae were treated per session, and this procedure was repeated five times. The control group larvae received a comparable amount of acetone. Once the extract was completely absorbed, the larvae were moved to their diet for ongoing monitoring. Over 8 days, the mortality of the larvae was noted every 24 hours. Larvae were classified as deceased if they showed no movement when gently nudged with forceps. Following 24 hours of exposure, the larvae were shifted to untreated, fresh cauliflower leaves. Each treatment was carried out with six repetitions.

Measurement Studies

Calibration of the microscope was performed using a 10x lens for low power, and 45x and 60x for higher power. The ocular lens (10x) was replaced with an ocular micrometre, and a stage micrometre was placed on the microscope stage. Proper calibration and focusing were achieved using the microscope. The readings and average values for micrometre divisions were recorded after aligning and focusing the scales.

Results

In this study, the life cycle of the Diamondback Moth called *P. xylostella* was observed, and the adverse effect of andrographolide, a terpenoid compound from *A.paniculata* (nilavembu), on the pupal-adult transformation was investigated (Figure 3).

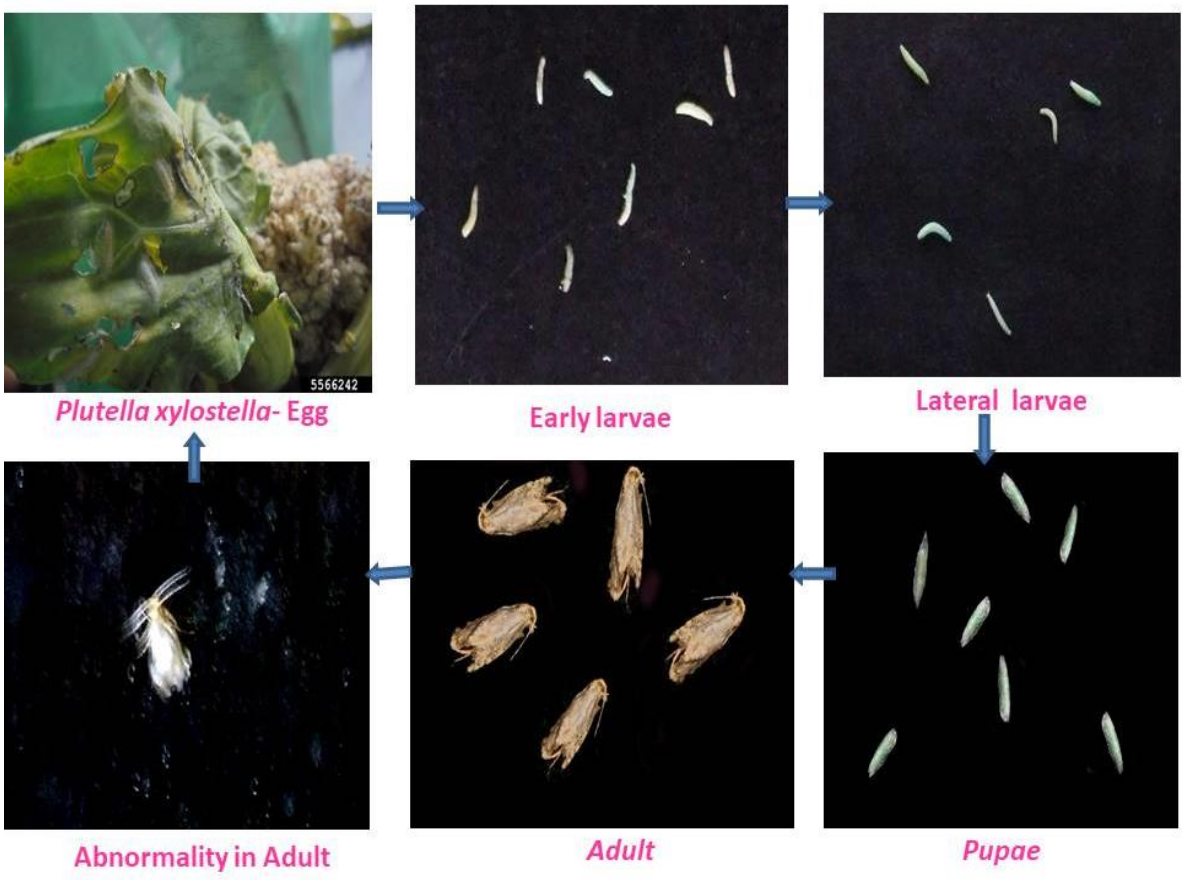

Figure 3. The life cycle of Diamond Back Moth (*Plutella xylostella*)

GC-MS Analysis

The phytochemical components of *A. paniculata* were explored by Ali et al. The phytochemicals that have been identified include methyl 8,10-octadecadiynoate, l-9-octadecenoic acid, stigmasterol, phenanthrene carboxylic acid, 13,15-octacosadiyne, and octadecanoic acid (Table 1) [18].

Table 1. Detection of Phytochemical Components of *A. paniculata* using GCMS Analysis [18]

S. No	Retention Time	Area %	Molecular Weight	Name of the Compounds
1	18.122	1.21	284	Octadecanoic acid
2	32.211	3.23	412	Stigmasterol
3	29.162	19.12	318	Phenanthrenecarboxylic acid
4	16.212	6.32	282	l-9-Octadecenoic acid
5	24.132	5.23	386	13,15-Octacosadiyne
6	27.132	1.23	290	Methyl 8,10-octadecadiynoate

FTIR Analysis

The active functional groups in the plant extract were reported by Ikhmal et al. using FTIR spectroscopic characterization. The explored peaks can be attributed to the stretching of carboxylic acids (3361.00 cm^{-1}), alkanes (2910.25 cm^{-1}), nitro compounds (1539.98 cm^{-1}), alcohols, carboxylic acids, esters, and ethers (1080.11 cm^{-1}) [19].

Diamondback Moth Life Cycle

The eggs of the diamondback moth are small, spherical, yellowish-white, and usually laid on the underside of lower leaves or stalks either singly or in clusters of two or three. Depending on the temperature, egg hatching takes place in 5 to 10 days. Larvae of the diamondback undergo four instars or growth stages. Earlier instars mine within the tissue of leaves, whereas later instars feed on the undersides of mature plant leaves or the heart leaves of young plants. Depending on temperature, larvae develop over 10 days to 4 weeks. Pupae grow inside a thin, open lacework cocoon that is delicately spun and bound to stems and leaves. Within seven to fifteen days, adults emerge. Adult moths are slender, about a third of an inch (8mm) long, greyish-brown, and have folded wings that flare outward and upward at the posterior ends. The folded forewings of the male form a row of three diamond-shaped yellow spots. Adult males live for about 12 days, while adult females live for about 16 days. Females lay eggs for approximately 10 days. Moths are slow fliers that are frequently carried by the wind. In temperate regions, the adult stage is the overwintering stage.

Effect of Andrographolide on Pupal-Adult Transformation

The study investigated the impact of topically applying andrographolide on the normal pupal-adult transformation in Diamondback Moths (Table 2).

Out of 15 early larvae that received andrographolide, 13% died within 24 hours, with a mortality rate increasing to 72% after 96 hours. Among 15 lateral larvae treated with andrographolide, 20% died within 24 hours, increasing to a 66% mortality rate after 120 hours. All 25 pupae treated with andrographolide had a 0% mortality rate at 48 hours, but a mortality rate of 80% was observed after 72 hours. Only one pupa successfully transformed into an adult, which exhibited morphological differences, notably a black spot on the head region.

Table 2. Mortality Rate of Andrographis *paniculata* on DBM Early Larvae, Lateral Larvae, and Pupae

S.No	Stage	Duration of Treatment	Mortality rate
1	Early larvae	24hrs	13%
2		48hrs	26%
3		72hrs	46%
4		96hrs	72%
5	Lateral larvae	24hrs	20%
6		48hrs	33%
7		72hrs	53%
8		96hrs	53%
9		120hrs	66%
10		144hrs	72%
11	Pupae	24hrs	0%
12		48hrs	0%
13		72hrs	80%

These results suggest that andrographolide application has a considerable outcome on the mortality and transformation of Diamondback Moth larvae and pupae, influencing their development into adults and resulting in morphological abnormalities. Further studies may elucidate the potential applications of *A. Paniculata* in pest management strategies.

Discussion

The research presented in this study centred on investigating the effects of *A.paniculata* (Nilavembu) plant extract on the metamorphosis of the Diamond Back Moth (DBM), *P. xylostella.* Phytochemicals such as methyl 8,10-octadecadiynoate, l-9-octadecenoic acid, stigmasterol, octadecanoic acid, phenanthrene carboxylic acid and 13,15-octacosadiyne were found in *A. paniculata* by GCMS analysis [18]. Active functional groups were reported by FTIR spectroscopy, which included nitro compounds (1539.98 cm^{-1}), alkanes (2910.25 cm^{-1}), carboxylic acids (3361.00 cm^{-1}), and a combination of alcohols, esters, and ethers (1080.11 cm^{-1}) [19]. The focus was particularly on how this plant extract influenced the transition from pupal to adult stage in DBM. The study encompassed various stages, including collecting, rearing, and observing the DBM life cycle in controlled laboratory conditions. The methodology involved collecting adult DBMs and their larvae from cauliflower plants in the Yercaud Hills. The DBMs were then reared in the laboratory under controlled conditions, and a crude plant extract was obtained from *A.paniculata* using the soxhlet extraction technique. This plant extract was applied topically to newly hatched larvae, while a carrier solvent (acetone) treatment was given to the control larvae. The purpose of this experiment was to determine if the normal pupal-to-adult transition of DBM was hindered by the application of the *A.paniculata* plant extract. Kalmegh (*A. paniculata*) contains a potent bioactive compound called andrographolide, which exhibits insecticidal and anti-feedant properties [20].

When compared to the control group, the results showed significant differences in morphology and larval mortality among emerging adults treated with the plant extract. This suggests that the *A. paniculata* plant extract has a notable impact on the development of *P. xylostella*. The comprehensive investigation of DBM's life cycle, including the various stages such as eggs, larvae, pupae, and adults, further supports these findings. According to Ramya et al. [21], crude methanol extract of *A. paniculata* has a mortality rate of all instars for *Helicoverpa armigera* larvae, with a higher

ED50 required to kill higher instars. The metabolism of *Parthenium hysterophorus L.* was also found to be affected by the phytotoxic effects of *A. paniculata* extract [22]. The application of andrographolide powder as an antifeedant led to a reduction in digestive enzyme activity within the midgut of *P. xylostella* (L.) larvae [8].

Baliyarsingh et al. [23] demonstrated that the insecticidal and repellency properties of *A. paniculata* leaf extracts are effective against *Tribolium castaneum*, with higher mortality rates observed as both the duration of exposure and the concentration of the solvent extracts increased. The insecticidal and antimicrobial properties of *A.paniculata* leaf extracts prepared using a hot-wet extraction method with five different solvents [24]. They found that the propanolic extract had an LD50 value of 63.27 mg/g after 10 days of treatment. Additionally, the aqueous (82%) and isopropanol extracts showed significant antibiosis effects. Edwin et al. [25] treated third, fourth, and fifth instar larvae of *Spodopteralitura* with varying concentrations of andrographolide (3, 6, and 9 ppm), resulting in decreased Relative Growth Rate (RGR), Relative Consumption Rate (RCR), Efficiency of Conversion of Ingested food (ECI), and Efficiency of Conversion of Digested food (ECD), while showing an adverse increase in Approximate Digestibility (AD). The treatment significantly inhibited digestive enzyme activity compared to the control and notably increased larval mortality. Revathi, [26] evaluated *A.paniculata* for its antifeedant, larvicidal, and adulticidal activities against *Henosepilachna vigintioctopunctata*, finding that isolated compounds stigmasterol and β-sitosterol exhibited significant antifeedant effects, with stigmasterol showing higher efficacy at 1000 ppm (67.83% phagodeterrency) than β-sitosterol (55.87%). Stigmasterol also caused the highest larval (76%) and adult (84%) mortality at 1000 ppm, indicating that phytosterols from *A. paniculata*, alongside andrographolide, offer promising pest control potential. The efficacy of extracts from different parts of jimsonweed (*Datura stramonium L.*) as larvicides and oviposition deterrents against *P.xylostella* (diamondback moth), finding that the flower extract exhibited a potent larvicidal activity with a 63% mortality rate against *P. xylostella* larvae [27]. The antifeedant activity of *Lampides boeticus* larvae when exposed to various concentrations of Andrographolide-based formulations [28]. The study found that formulation A, consisting of 70% Andrographolide, 20% Neem oil, and 10% Triton X-100, at a 7% concentration, exhibited the highest larval feeding inhibition, achieving a 93.05% reduction in feeding.

These observations open up possibilities for employing *A.paniculata* extract in pest control strategies within agricultural settings. The altered morphology and increased mortality among DBMs treated with the plant extract signify its potential as an effective natural biopesticide. Further research could delve into the mechanisms behind these effects, exploring the specific compounds within the plant extract [29-31] that are responsible for the observed variations in DBM development. Additionally, assessing the broader ecological impact and potential applicability in integrated pest management strategies would be valuable for optimizing the utilization of *A.paniculata* in sustainable pest control practices.

Conclusion

In conclusion, this research elucidates the significant influence of *A.paniculata* plant extract on the metamorphosis of *P. xylostella*, the Diamond Back Moth (DBM). The study's findings, highlighting distinct variations in morphology and increased larval mortality among DBMs treated with the plant extract, underscore the potential of *A.paniculata* as a natural biopesticide. The altered development observed in DBMs suggests its promise for integration into pest management strategies

within agricultural contexts, providing a potential eco-friendly solution to combatting this notorious agricultural pest. Further investigations into the specific bioactive compounds and broader ecological implications are warranted to unlock the full potential of *A.paniculata* in sustainable pest control.

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Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

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