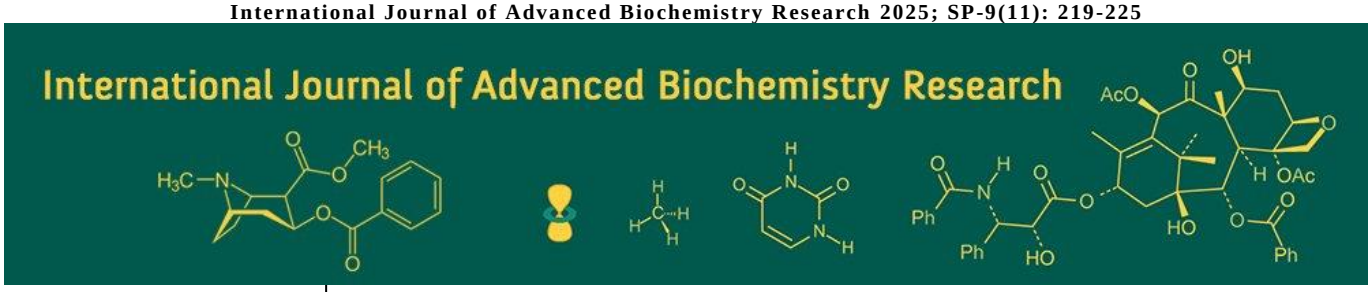

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(11): 219-225
www.biochemjournal.com
Received: 18-09-2025
Accepted: 21-10-2025

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Assessment of antiviral medicinal plant extracts against grasserie disease and their impact on biochemical and hematological parameters in silkworm (*Bombyx mori* L.)

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i11Sc.6221

Abstract
The present investigation assessed the effectiveness of three medicinal plant extracts—*Aloe vera*, *Phyllanthus niruri*, and *Andrographis paniculata*—in controlling Grasserie disease in *Bombyx mori* L., which is caused by the *Bombyx mori* nucleopolyhedrovirus (*Bm*NPV)**.** Among the evaluated treatments, *Aloe vera* exhibited the most significant positive impact on cocoon characteristics. The highest cocoon weight (1.85 g) was obtained in treatment T_1 (*Aloe vera* + *Bm*NPV)**,** followed by the control (T_5, distilled water) with 1.81 g and T_2 (*Phyllanthus niruri* + *Bm*NPV) with 1.75 g. The lowest value (1.25 g) was recorded in T_4 (*Bm*NPV at 1×10^5 POB ml^{-1}). Similar trends were evident in shell weight and shell ratio, with T_1 performing best, followed by T_5 and T_2, while T_4 recorded the minimum values. Disease occurrence in the $FC_1\times FC_2$ bivoltine silkworm double hybrid was markedly reduced following treatment with the medicinal plant extracts compared to the infected control. The total hemocyte count (THC) in T_1 showed a significant rise from 3205 ml^{-1} on day 1 to 5924 ml^{-1} on day 5, followed by a decline to 4256 ml^{-1} by day 6, which was comparable to the control (T_5). A similar pattern was observed in protein concentration, where T_1 registered the highest level (92.6 mg ml^{-1}) on day 6, slightly exceeding the control (91.3 mg ml^{-1}). Conversely, T_4 exhibited the lowest protein content (12.3 mg ml^{-1}). Overall, *Aloe vera* displayed superior antiviral potential against Grasserie disease compared to *Phyllanthus niruri* and *Andrographis paniculata*. These results indicate that *Aloe vera* extract could be effectively utilized as a component of bed disinfectants to prevent viral infections during silkworm rearing.

Keywords: *Aloe vera*, *Bombyx mori* L., Grassrie, medicinal plant extracts, pathogen inoculation, silkworm

Introduction
The mulberry silkworm, *Bombyx mori* L., plays a pivotal role in the global silk industry and serves as a vital source of income and foreign exchange for several silk-producing countries (Krishnaswami *et al.*, 1973; Isaiarasu *et al.*, 2011) [7, 3]. In tropical regions where silkworm rearing is practiced throughout the year, the insects are frequently exposed to a range of pathogenic infections, making disease management one of the major challenges in sericulture (Samson *et al.*, 1998; Rajeswari and Isaiarasu, 2004; Isaiarasu *et al.*, 2011) [18, 16, 3]. These diseases collectively account for considerable economic losses, often reducing cocoon yields by 30-40% (Chandrasekharan *et al.*, 2006) [25]. Among them, Grasserie, a viral disease caused by the *Bombyx mori* nucleopolyhedrovirus (*Bm*NPV), remains the most destructive, persisting year-round under tropical climatic conditions (Sivaprakasam, 1999; Latha *et al.*, 2011; Manjunath *et al.*, 2020) [20, 8, 11].
Medicinal and aromatic plants, known for their abundance of bioactive phytochemicals, have long been utilized in traditional medicine for their therapeutic properties. These plants produce a wide array of secondary metabolites, which, though not directly linked to primary metabolism, play key roles in ecological interactions and serve as natural defense agents against pathogens (Jain *et al.*, 2004; Isaiarasu *et al.*, 2011) [5, 3]. The antimicrobial and antiviral nature of these compounds offers promising applications in silkworm health management, particularly in mitigating viral infections such as *Bm*NPV.

Apart from inhibiting viral replication, these botanicals are also reported to enhance growth, immunity, and overall vitality in silkworms (Latha *et al.*, 2011) [8]. Several earlier studies have confirmed the antiviral and antimicrobial efficacy of various plant-derived substances, underscoring their potential for sustainable disease control in sericulture (Gouda, 1991; Sridevi, 2003; Murthy, 2004; Shubha, 2005; Manimegalai and Chandramohan, 2006) [1, 21, 14, 19, 10].

The growth and physiological development of insects are largely governed by biochemical constituents such as carbohydrates, proteins, and lipids (Ito and Horie, 1959; Wyatt and Kalf, 1956; Latha *et al.*, 2011; Ananda Kumar and Michael, 2012) [4, 22, 8, 23]. In particular, haemolymph proteins are crucial for transport processes, enzymatic functions, and immune responses, with their synthesis influenced by both genetic and hormonal regulation (Hurliman and Chen, 1974; Latha *et al.*, 2011) [2, 8]. The haemolymph, an extracellular fluid analogous to blood in vertebrates, performs multiple physiological roles including nutrient transport, excretion, moulting, metamorphosis, and defense mechanisms such as phagocytosis and encapsulation (Gad and Alzahofi, 2010; Nazar *et al.*, 2020) [26, 15].

The present study was undertaken to evaluate the antiviral potential of three medicinal plant extracts—*Aloe vera***,** *Phyllanthus niruri***,** and *Andrographis paniculata*—against Grasserie disease in *B. mori*. These plants were selected based on their previously reported antiviral properties, easy availability, and suitability for eco-friendly disease management. Furthermore, the study assessed the influence of these plant extracts on protein activity and haemolymph parameters in *Bm*NPV-infected silkworms. The experimental work was conducted at Sri Krishnadevaraya University, Anantapur, with the overarching objective of identifying effective, plant-based antiviral agents for sustainable disease management in sericulture.

Materials and Methods

Preparation of Plant Extracts

Extracts from three medicinal plants—*Aloe vera*, *Phyllanthus niruri*, and *Andrographis paniculata*—were prepared with minor procedural modifications. Fresh plant materials were initially shade-dried to retain their bioactive constituents and subsequently ground into a fine powder using an electric blender. All extraction procedures were carried out at Sri Krishnadevaraya University, Anantapur. For each plant species, 10 g of the powdered material was soaked in 100 ml of distilled water for six hours to facilitate the release of active compounds. The mixture was then subjected to continuous stirring for one hour on a magnetic stirrer to ensure complete extraction.

After stirring, the resulting mixture was filtered through Whatman filter paper to separate the solid residues. The filtrate was stored at 4 °C for subsequent experimental use, following the method described by Manjunath *et al.* (2020) [11]. To obtain the working solution, each extract was diluted to a 5% concentration using double-distilled water, ensuring consistency across all treatments. These prepared plant extracts were later employed to evaluate their efficacy in managing Grasserie disease in *Bombyx mori*.

Treatment Details

T_1: Aloe vera + *Bm*NPV of 1×10^5 POB ml^{-1}
T_2: *Phyllanthus niruri* + *Bm*NPV of 1×10^5 POB ml^{-1}
T_3: *Andrographis paniculata* + *Bm*NPV of $1x10^5$ POB ml^{-1}
T_4: *Bm*NPV of $1x10^5$ POB ml^{-1}
T_5: Distilled water control

Schedule of Treatment

To evaluate the antiviral efficacy of the selected medicinal plant extracts, five treatment groups (T_1-T_5) were established. The extracts were applied to fresh mulberry leaves, which were subsequently fed to *Bombyx mori* larvae on the first day of the fourth and fifth instar stages. The experimental larvae belonged to the $FC_1\times FC_2$ bivoltine double hybrid. Observations were made to assess the impact of the treatments on cocoon characteristics, protein concentration, and total haemocyte count (THC).

For reliability and reproducibility, the experiment was conducted in three replications, each consisting of 100 larvae, following the standardized method described by Shubha (2005) [19]. This experimental design provided a strong statistical basis for evaluating the antiviral potential of the medicinal plant extracts and their influence on the physiological and biochemical responses of *B. mori* larvae infected with Grasserie disease.

Haemolymph Collection

Haemolymph samples were collected from silkworms belonging to all treatment groups (T_1-T_5) in pre-chilled test tubes to preserve sample stability and prevent coagulation. The collected haemolymph was immediately centrifuged at 1500 rpm for 10 minutes, and the resulting supernatant was carefully transferred into sterile microtubes. The samples were stored at-20 °C until further biochemical analysis, following the procedure outlined by Isaiarasu *et al.* (2011) [3].

Estimation of Total Soluble Proteins

The total soluble protein content in the haemolymph was quantified using the Lowry method (Lowry *et al.*, 1951) [9], employing bovine serum albumin (BSA) as the standard reference. The estimation procedure followed the protocol described by Ananda Kumar and Michael (2012) [23] to ensure accuracy and consistency across all samples.

Counting the Total Haemocytes (THC)

For the estimation of total haemocyte count (THC)**,** 1 ml of phosphate-buffered saline (PBS) was added to each haemolymph sample as an anticoagulant. A 10 µl aliquot of the diluted haemolymph was then loaded onto a Neubauer hemocytometer, and haemocytes were counted under a stereo microscope at 40× magnification. The method employed was based on the standard procedure reported by Nazar *et al.* (2020) [15].

Cocoon Weight (g)

Ten cocoons were randomly selected from each replication on the sixth day after spinning. Each cocoon was weighed individually, and the average cocoon weight was calculated. This procedure was performed according to the method described by Ananda Kumar and Michael (2012) [23] and Kapoor *et al.* (2022) [6].

Shell Weight (g)

For determining shell weight, ten cocoons from each replication were randomly chosen. The pupa was carefully removed, and the remaining cocoon shells were weighed. The mean shell weight was calculated as per the procedures outlined by Ananda Kumar and Michael (2012) [23] and Kapoor *et al.* (2022) [6].

Shell Ratio (%)

The shell ratio was computed using the ratio of shell weight to total cocoon weight, expressed as a percentage, following Kapoor *et al.* (2022) [6].

$$\text{Shell Ratio} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

Pupal Weight (g)

For the determination of pupal weight, ten cocoons were randomly selected from each replication. The pupa was separated from the cocoon, and its weight was measured individually. The average pupal weight was then calculated in accordance with the method described by Kapoor *et al.* (2022) [6].

Disease Incidence (%)

Throughout the rearing period, the numbers of healthy and diseased larvae were recorded for each treatment. The disease incidence was determined using the following formula, as suggested by Kapoor *et al.* (2022) [6].

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased larvae}}{\text{Total No. of larvae}} \times 100$$

Results and Discussion

Influence of Plant Extracts on Cocoon Parameters

The application of medicinal plant extracts significantly influenced cocoon-related traits such as cocoon weight**,** shell weight, and shell ratio. Among the different treatments, the *Aloe vera* + *Bm*NPV group **(**T_1**)** recorded the highest cocoon weight (1.85 g), followed by the distilled water control (T_5) at 1.81 g, and *Phyllanthus niruri* + *Bm*NPV (T_2) at 1.75 g. The lowest cocoon weight (1.25 g) was observed in the *Bm*NPV-only treatment (T_4) (Table 1). Similar trends were observed for shell weight and shell ratio, where T_1 exhibited superior performance, followed by T_5, T_2, and T_3, while T_4 consistently showed the lowest values. These results highlight the protective and growth-promoting potential of *Aloe vera*, which enhanced cocoon production even under viral infection stress.

Table 1: Impact of various medical plant treatments on the rearing performance of FC1×FC2 bivoltine double hydrid mulberry silkworm (*Bombyx mori* L.)

Treatments	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Pupa weight (g)	Disease incidence (%)
T_1: Aloe vera + *Bm*NPV of 1×10^5 POB ml^{-1}	1.85	0.41	22.16	1.44	23.6
T_2: *Phyllanthus niruri* + *Bm*NPV of 1×10^5 POB ml^{-1}	1.75	0.38	21.78	1.37	56.9
T_3:*Andrographis paniculata* + *Bm*NPV of $1x10^5$ POB ml^{-1}	1.63	0.32	19.63	1.31	75.2
T_4: *Bm*NPV of $1x10^5$ POB ml^{-1}	1.25	0.24	19.28	1.01	78.4
T_5: Distilled water control	1.81	0.40	22.21	1.41	5.4

The findings of this study are in line with earlier research. Manoharan (1996) [12] reported an increase in cocoon weight from 1.50 g to 1.68 g and in shell weight from 0.28 g to 0.312 g when aqueous extracts of *Psoralea corylifolia*, *Tribulus terrestris*, *Acanthospermum hispidum*, *Caesalpinia coriaria*, and Bougainvillea antiviral protein were administered to the PM × NB4D2 hybrid, compared with the control values (1.45 g and 0.280 g, respectively). This enhancement was attributed to the dual role of these botanicals in suppressing *Bm*NPV infection and improving silk yield and quality. Similarly, Sivaprakasam (1999) [20] demonstrated that Bougainvillea antiviral protein purified from *B. spectabilis* conferred strong protection against *Bm*NPV while simultaneously enhancing silk production in PM × NB4D2 hybrids. Supporting these observations, Latha *et al.* (2011) [8] also reported improved cocoon quality following the administration of plant-based antiviral formulations.

Collectively, these results reinforce the efficacy of plant-derived bioactive compounds, particularly *Aloe vera*, in promoting silkworm health and productivity by mitigating viral infection effects and enhancing cocoon parameters.

Influence of Plant Extracts on Disease Incidence (%)

The administration of medicinal plant extracts significantly reduced the incidence of Grasserie disease in the $FC_1\times FC_2$ bivoltine silkworm double hybrid. All treatment groups showed a marked decline in disease incidence compared to the untreated control, indicating the antiviral potential of the tested botanicals. The lowest disease incidence was observed in the distilled water control (T_5), followed closely by the *Aloe vera* + *Bm*NPV treatment (T_1), which recorded 23.6% incidence. Moderate disease levels were observed in the *Phyllanthus niruri* + *Bm*NPV group (T_2) with 56.9%**,** while the *Andrographis paniculata* + *Bm*NPV group (T_3) exhibited a higher incidence of 75.2%. The highest infection rate (78.4%) occurred in the *Bm*NPV-only treatment (T_4) (Table 1; Figure 1).

These observations confirm that *Aloe vera* is particularly effective in suppressing Grasserie disease, exhibiting stronger antiviral activity compared to the other two plant extracts and the virus-only control. The variation in antiviral responses among the treatments suggests that the bioactive constituents of these plants may differ in their ability to inhibit viral replication or boost host immunity. Such results emphasize the potential application of botanical formulations as part of an integrated disease management strategy in sericulture (Ram, 2000) [17], with *Aloe vera* emerging as a highly promising candidate for mitigating viral infections and enhancing silkworm health.

Earlier investigations support these findings. Sridevi (2003) [21] and Manjunath *et al.* (2020) [11] reported a substantial reduction in the incidence of diseases such as Grasserie and Flacherie in silkworm hybrids **(**CSR2×CSR4 and PM×CSR2) when supplemented with medicinal plant extracts, compared to untreated controls. These studies, in agreement with the present results, demonstrate that plant-based antiviral treatments can effectively reduce disease prevalence and improve both health and productivity in silkworms across different hybrids. The consistent antiviral performance of medicinal plants underscores their potential as eco-friendly, sustainable alternatives for disease management in modern sericulture practices.

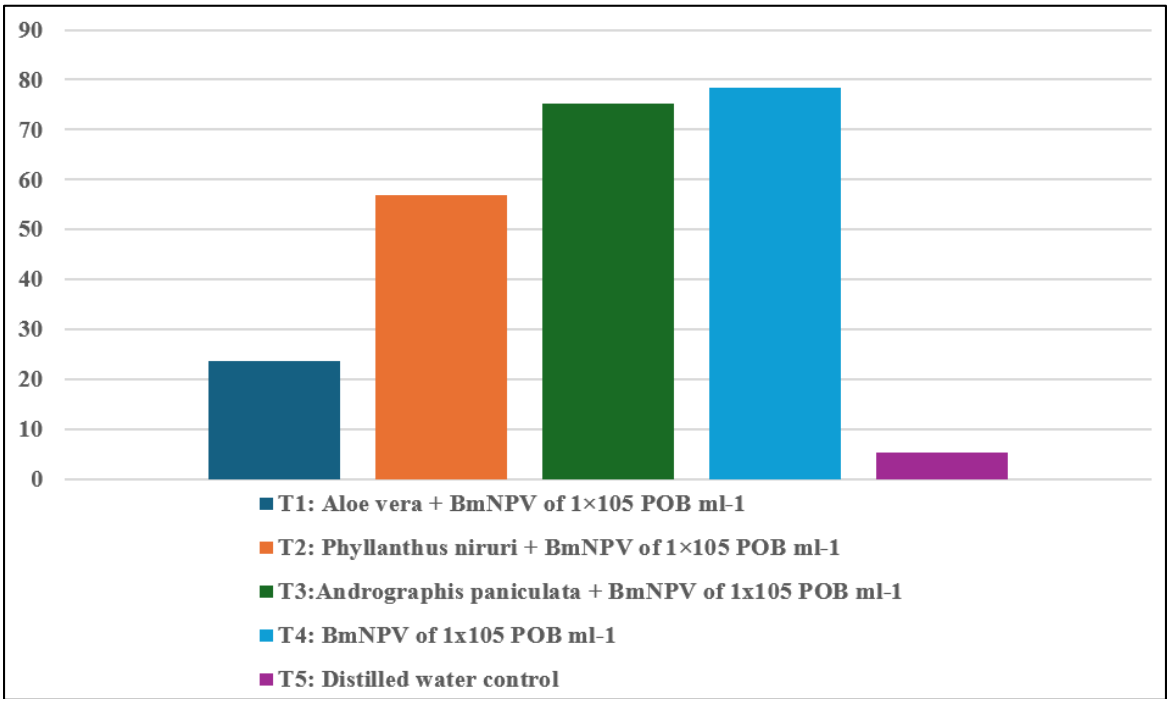

Fig 1: Disease incidence (%) during the rearing of $FC_1 \times FC_2$ bivoltine double hybrid after administration of T_1 to T_5 treatments.

Influence of Plant Extracts on Total Haemocyte Count (THC)

The administration of medicinal plant extracts had a significant effect on the total haemocyte count (THC) in silkworms infected with *Bm*NPV. In the *Bm*NPV-only treatment (T_4), THC initially increased from 3,194 ml^{-1} on day 1 to 5,923 ml^{-1} by day 3 post-inoculation. However, from day 4 onward, a sharp decline was observed, with THC dropping to 194 ml^{-1} by day 6. In contrast, the control group (T_5) exhibited a gradual rise in THC from 3,154 ml^{-1} on day 1 to 6,058 ml^{-1} by day 5, followed by a slight decrease to 4,320 ml^{-1} during the spinning stage (Table 2; Figure 2).

The *Aloe vera* + *Bm*NPV treatment (T_1) displayed a pattern similar to the control, with THC increasing from 3,205 ml^{-1} on day 1 to 5,924 ml^{-1} by day 5, before declining to 4,256 ml^{-1} on day 6, closely approximating the control values. This indicates that *Aloe vera* helped maintain haemocyte levels in infected larvae, likely due to its antiviral and immunomodulatory properties. Treatments T_2 (*Phyllanthus niruri* + *Bm*NPV) and T_3 (*Andrographis paniculata* + *Bm*NPV) exhibited similar trends, though they ranked third and fourth, respectively, in maintaining THC levels.

These observations are consistent with previous reports. Ananda Kumar and Michael (2011) [23] and Nazar *et al.* (2020) [15] noted that silkworm larvae infected with pathogens such as *Bacillus thuringiensis* showed marked reductions in THC, with Flacherie-infected larvae experiencing a 15.3% decline compared to healthy controls. Such decreases in haemocyte counts are indicative of physiological stress and weakened immunity, often resulting from reduced feeding and metabolic activity in infected larvae (Balavenkatasubbalah and Sivaprasad, 2014; Nazar *et al.*, 2020) [24, 15].

Overall, these results underscore the potential of medicinal plant extracts in enhancing immune function and sustaining haemocyte levels, thereby improving resistance to viral infections and supporting healthier growth in *B. mori*.

Table 2: Impact of various medicinal plant treatments on total haemocyte count (THC).

Treatments	1st day (THC ml^{-1})	2nd day (THC ml^{-1})	3rd day (THC ml^{-1})	4th day (THC ml^{-1})	5th day (THC ml^{-1})	6th day (THC ml^{-1})
T_1: Aloe vera + *Bm*NPV of 1×10^5 POB ml^{-1}	3205	4329	5009	5710	5924	4256
T_2: *Phyllanthus niruri* + *Bm*NPV of 1×10^5 POB ml^{-1}	3246	4768	5376	4918	4736	3675
T_3:*Andrographis paniculata* + *Bm*NPV of 1x10^5 POB ml^{-1}	3151	5047	5864	4867	4592	2961
T_4: *Bm*NPV of 1x10^5 POB ml^{-1}	3194	5673	5923	3526	549	194
T_5: Distilled water control	3154	4157	4985	5927	6058	4320

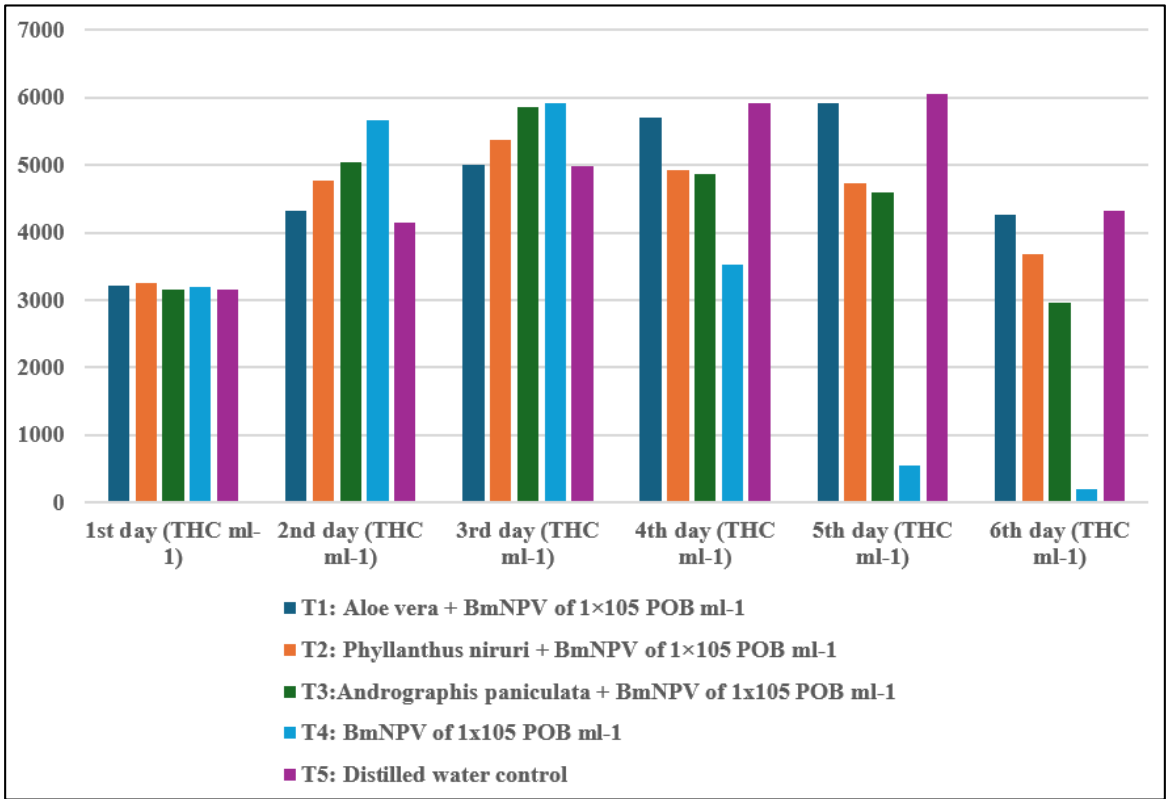

Fig 2: Effect of different medicinal plant treatments on total hemocyte count.

Estimation of Total Soluble Proteins in the Haemolymph

The total soluble protein content in the haemolymph of bivoltine hybrid silkworms exhibited a steady increase from the first day through to the spinning stage across all treatment groups. This age-dependent rise in protein levels is consistent with previous findings reported by Ananda Kumar and Michael (2012) [23], reflecting the progressive accumulation of haemolymph proteins during larval development.

Among the treatments, the *Aloe vera* + *Bm*NPV group (T_1) recorded the highest protein concentration on day six, reaching 92.6 mg ml^{-1}, closely followed by the healthy control (T_5) at 91.3 mg ml^{-1}. Treatments T_2 (*Phyllanthus niruri* + *Bm*NPV) and T_3 (*Andrographis paniculata* + *Bm*NPV) also exhibited appreciable protein levels, though these were lower than T1 and T_5. The *Bm*NPV-only treatment (T_4) showed the lowest protein concentration, with a markedly reduced value of 12.3 mg ml^{-1} (Table 3; Figure 3).

These results indicate that *Aloe vera* extract effectively maintained higher haemolymph protein levels even in the presence of *Bm*NPV infection, closely mirroring those of healthy larvae. The pronounced decline in protein concentration in the virus-only group underscores the detrimental effect of *Bm*NPV on silkworm physiology. Conversely, the ability of Aloe vera and other medicinal plant extracts to support protein synthesis and bolster immune function highlights their potential as protective agents in silkworm rearing.

Table 3: Impact of different medicinal plant treatments on hemolymph (mg ml^{-1})

Treatments	1st day	2nd day	3rd day	4th day	5th day	6th day
T_1: Aloe vera + *Bm*NPV of 1×10^5 POB ml^{-1}	33.4	46.8	68.2	79.4	86.7	92.6
T_2: *Phyllanthus niruri* + *Bm*NPV of 1×10^5 POB ml^{-1}	31.8	42.4	56.4	65.1	72.4	75.2
T_3:*Andrographis paniculata* + *Bm*NPV of 1x10^5 POB ml^{-1}	32.0	43.7	55.1	63.4	69.9	71.4
T_4: *Bm*NPV of 1x10^5 POB ml^{-1}	31.6	33.9	35.7	34.7	15.1	12.3
T_5: Distilled water control	32.5	47.8	71.3	80.7	86.4	91.3

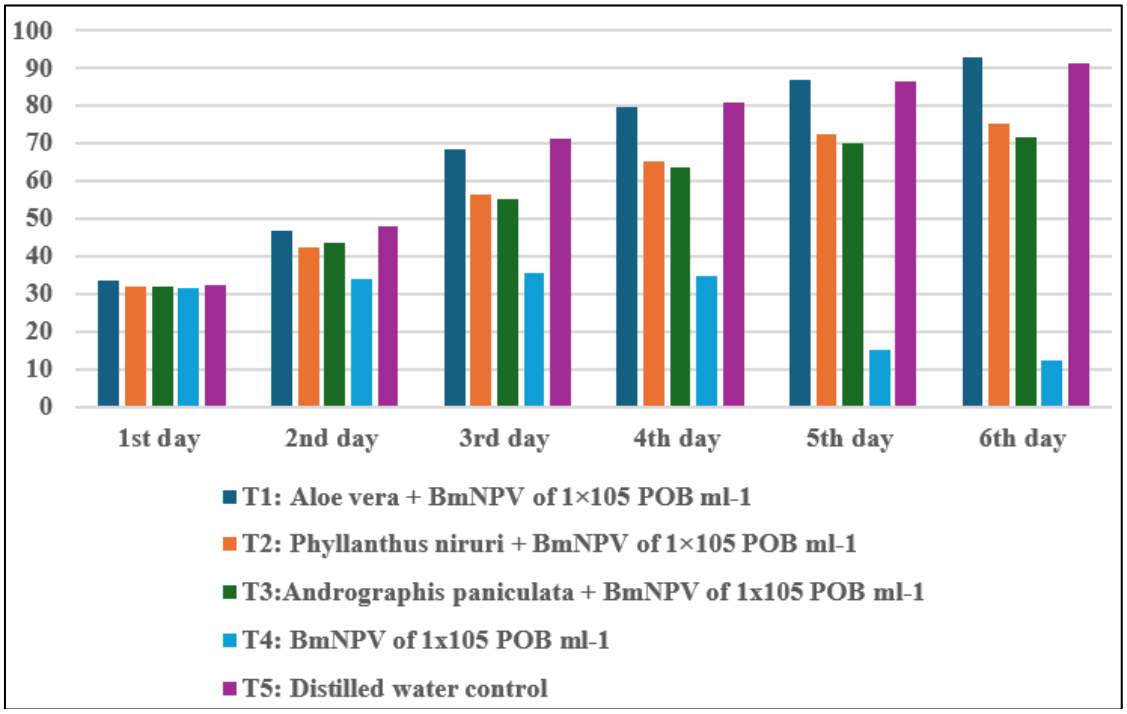

Fig 3: Effect of various medicinal plant treatments on haemolymph protein levels (mg ml^{-1})

Conclusion

Among the three medicinal plants tested—*Aloe vera*, *Phyllanthus niruri*, and *Andrographis paniculata*—*Aloe vera* demonstrated the highest antiviral efficacy against Grasserie disease in *Bombyx mori*, while also significantly enhancing key cocoon parameters. All tested plant extracts positively influenced total haemocyte count (THC) and haemolymph protein levels, contributing to improved silkworm health. Based on these findings, *Aloe vera* shows promise as a natural additive for bed disinfectants and can be effectively incorporated into integrated strategies for the management of viral diseases in silkworm rearing.

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