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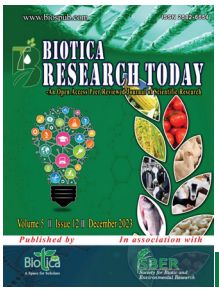

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Evaluation of Influenced Antiviral Medicinal Plant Extracts for the Control of Grasserie Disease and Estimation of Biochemical and Hematological Changes in Silkworm, *Bombyx mori* L.

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Abstract

The current study evaluates the efficacy of three medicinal plant extracts, *viz*., *Aloe vera*, *Phyllanthus niruri* and *Andrographis paniculata*, in mitigating Grasserie disease in *Bombyx mori* L., a disease caused by the *Bombyx mori* nucleopolyhedrovirus (*Bm*NPV). Among the tested treatments, *Aloe vera* exhibited the most significant positive effects on cocoon parameters. The highest cocoon weight of 1.85 g was recorded in the treatment group T_1 (*Aloe vera* + *Bm*NPV), followed by the control group T_5 (distilled water) with 1.81 g and T_2 (*Phyllanthus niruri* + *Bm*NPV) with 1.75 g. The lowest cocoon weight of 1.25 g was observed in T_4 (*Bm*NPV at 1×10^5 POB ml^{-1}). Similar trends were observed for shell weight and shell ratio, where T_1 ranked highest, followed by T_5 and T_2, with T_4 showing the lowest values. Disease incidence in the $FC_1\times FC_2$ bivoltine silkworm double hybrid was significantly reduced following the administration of medicinal plant extracts compared to the control. Specifically, total hemocyte count (THC) in T_1 increased significantly from 3205 ml^{-1} to 5924 ml^{-1} from day 1 to day 5 post-treatment, then decreased to 4256 ml^{-1} by day 6, which approximated the control (T_5). Protein levels followed a similar pattern, with T_1 recording the highest value of 92.6 mg ml^{-1} on day 6, compared to 91.3 mg ml^{-1} in the control. T_4 exhibited the lowest protein concentration at 12.3 mg ml^{-1}. Overall, *Aloe vera* demonstrated superior antiviral activity against Grasserie disease compared to *Phyllanthus niruri* and *Andrographis paniculata*. The findings suggest that *Aloe vera* may be effectively incorporated into bed disinfectants as a preventive measure against viral diseases in silkworm rearing.

Keywords: *Aloe vera*, *Bombyx mori* L., Grassrie, Medicinal plant extracts, Pathogen inoculation, Silkworm

Introduction

The mulberry silkworm, *Bombyx mori* L., holds immense economic significance as a key contributor to the silk industry, serving as a major source of foreign exchange for many silk-producing nations (Krishnaswami *et al.*, 1973; Isaiarasu *et al.*, 2011). In tropical regions, where *B. mori* is reared continuously, silkworm populations are highly vulnerable to pathogenic infections, making disease management a critical challenge (Samson *et al.*, 1998; Rajeswari and Isaiarasu, 2004; Isaiarasu *et al.*, 2011). Various diseases contribute to substantial cocoon crop losses, estimated at 30-40% (Chandrasekharan *et al.*, 2006). Among these, Grasserie, a viral infection caused by the *Bombyx mori* nucleopolyhedrovirus (*Bm*NPV), is the most devastating, persisting year-round in tropical climates (Sivaprakasam, 1999; Latha *et al.*, 2011; Manjunath *et al.*, 2020).

Medicinal and aromatic plants are rich sources of natural bioactive compounds and have been traditionally utilized for health care in humans and animals. These plants produce secondary metabolites, which, while not directly involved in the plant's metabolic functions, often play a critical role in interactions with other organisms, frequently serving

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as biochemical defenses (Jain *et al.*, 2004; Isaiarasu *et al.*, 2011). The antimicrobial and antiviral properties of these biomolecules offer potential for use in silkworm disease management, particularly against *Bm*NPV, where they not only inhibit viral spread but also promote silkworm growth and development (Latha *et al.*, 2011). Previous studies have demonstrated that botanicals possess both antimicrobial and antiviral activities, making them valuable for enhancing silkworm health and reducing viral infections (Gouda, 1991; Sridevi, 2003; Murthy, 2004; Shubha, 2005; Manimegalai and Chandramohan, 2006).

Carbohydrates, proteins and lipids are essential components in the biochemical processes governing insect growth and development (Ito and Horie, 1959; Wyatt and Kalf, 1956; Latha *et al.*, 2011; Ananda Kumar and Michael, 2012). In particular, haemolymph proteins play a vital role in transport, enzyme activity and immune functions in insects, with their synthesis and regulation controlled by genetic and hormonal factors (Hurliman and Chen, 1974; Latha *et al.*, 2011). Haemolymph, the extracellular fluid in insects, facilitates key physiological functions, including mineral transport, moulting, excretion, metamorphosis and immune defense mechanisms such as phagocytosis and encapsulation (Gad and Alzahofi, 2010; Nazar *et al.*, 2020).

In the present study, we investigated the antiviral potential of three medicinal plants, *viz*., *Aloe vera*, *Phyllanthus niruri* and *Andrographis paniculata* against Grasserie disease in *B. mori*. These plants were selected based on their documented antiviral properties, local availability and potential efficacy in controlling *Bm*NPV. Additionally, the study explored the effects of these plant extracts on protein activity and haemolymph parameters in silkworms treated with *Bm*NPV. The experiment was conducted at Sri Krishnadevaraya University, Anantapur, aiming to identify effective plant-based interventions for managing viral diseases in sericulture.

Materials and Methods

Preparation of Plant Extracts

Three different plant's extracts from *Aloe vera*, *Phyllanthus niruri* and *Andrographis paniculata* were prepared with slight modifications. The medicinal plant samples were first shade-dried to preserve their bioactive compounds and then ground into a fine powder using an electric blender. This process was conducted at Sri Krishnadevaraya University, Anantapur. For each plant, 10 g of the powdered material was soaked in 100 ml of distilled water for six hours to extract the active compounds. The mixture was then stirred continuously for one hour using a magnetic stirrer to ensure thorough extraction.

Following the stirring process, the solution was filtered through Whatman filter paper to remove solid residues. The filtrates were stored at 4 °C for future use, as recommended by Manjunath *et al.* (2020). To prepare the working solution, the extract was diluted to a 5% concentration using double-distilled water, ensuring uniformity in treatment across the study. These extracts were subsequently utilized in experiments to evaluate their efficacy in controlling 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 1×10^5 POB ml^{-1}

T_4: *Bm*NPV of 1×10^5 POB ml^{-1}

T_5: Distilled water control

Schedule of Treatment

To assess the antiviral properties of the selected medicinal plants, treatments T_1 through T_5 were administered by applying the plant extracts to mulberry leaves, which were then fed to *Bombyx mori* larvae on the first day of their fourth and fifth instar stages. The larvae used in the study belonged to the $FC_1\times FC_2$ bivoltine hybrid. The effects of the treatments on cocoon parameters, protein levels and total haemocyte count (THC) were carefully monitored and recorded for subsequent analysis.

For accuracy and reproducibility, the experiment was conducted with three replications, each containing 100 larvae, following the procedure established by Shubha (2005). This setup ensured a robust statistical foundation for evaluating the efficacy of the medicinal plant extracts in controlling Grasserie disease and influencing the physiological responses of the larvae.

Haemolymph Collection

Haemolymph from silkworms across all treatments (T_1 to T_5) was collected in pre-chilled test tubes to maintain sample integrity. The samples were then centrifuged at 1500 rpm for 10 minutes, after which the supernatant was carefully transferred to storage tubes and preserved at -20 °C, following the protocol described by Isaiarasu *et al.* (2011).

Estimation of Total Soluble Proteins

The quantitative estimation of total soluble proteins in the haemolymph was performed using the Lowry method (Lowry *et al.*, 1951), with bovine serum albumin as the standard. This method was employed as per the procedures outlined by Ananda Kumar and Michael (2012).

Counting the Total Haemocytes (THC)

Before the haemocyte count, 1 ml of phosphate-buffered saline (PBS) was added as an anticoagulant to ensure accurate results. The total haemocyte count (THC) was measured using a Neubauer hemocytometer. A 10 µl sample was pipetted and the cells were counted under a stereo microscope at 40X magnification, following the methodology of Nazar *et al.* (2020).

Cocoon Weight (g)

Ten cocoons were randomly selected from each replication on the sixth day after spinning. The weight of each cocoon was recorded individually, and the average cocoon weight was calculated. This procedure was followed as per the methods described by Ananda Kumar and Michael (2012) and Kapoor *et al.* (2022).

Shell Weight (g)

For shell weight determination, 10 cocoons from each replication were randomly selected. The weight of the cocoon shell was measured after removing the pupa, following the guidelines of Ananda Kumar and Michael (2012) and Kapoor *et al.* (2022).

Shell Ratio (%)

The shell ratio was calculated based on the shell weight and cocoon weight using the following formula, as described by Kapoor *et al.* (2022).

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

Pupa Weight (g)

For determining pupal weight, 10 randomly selected cocoons from each replication were used. The pupa was removed from each cocoon, and its weight was recorded. The average pupal weight was calculated following the method of Kapoor *et al.* (2022).

Disease Incidence (%)

During the rearing process, the total numbers of healthy and diseased larvae were recorded in each treatment group. The disease incidence was calculated and expressed as a percentage of the total larvae affected, following the formula,

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

Results and Discussion

Influence of Plant Extracts on Cocoon Parameters

The application of medicinal plant extracts had a notable impact on cocoon parameters, particularly cocoon weight, shell weight and shell ratio. The highest cocoon weight was recorded in the T_1 treatment group (*Aloe vera* + *Bm*NPV), with an average of 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 was observed in the *Bm*NPV-only treatment (T_4) at 1.25 g (Table 1). In terms of shell weight and shell ratio, T_1 ranked the highest, followed by T_5, T_2 and T_3, while T_4 exhibited the lowest values. These results demonstrate the protective and growth-promoting effects of *Aloe vera* on silkworm cocoon production, even in the presence of *Bm*NPV infection.

Table 1: Impact of various medicinal plant treatments on the rearing performance of $FC_1 \times FC_2$ bivoltine double hybrid mulberry silkworms (*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 1×10^5 POB ml^{-1}	1.63	0.32	19.63	1.31	75.2
T_4: *Bm*NPV of 1×10^5 POB ml^{-1} (Inoculated Control)	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

Previous studies have corroborated these findings. Manoharan (1996) 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 *P. coryleifolia*, *T. terrestris*, *A. sumo*, *C. coriaria* and Bougainvillea antiviral protein were administered to the $PM\times NB_4D_2$ hybrid, compared to control values of 1.45 g and 0.280 g, respectively. This improvement in cocoon parameters was attributed to the dual role of these extracts in providing antiviral protection against *Bm*NPV and enhancing silk yield and quality. Similarly, Sivaprakasam (1999) documented that the introduction of Bougainvillea antiviral protein purified from *B. spectabilis* into $PM\times NB_4D_2$ crossbreeds not only offered protection against *Bm*NPV but also improved silk yield and quality. These earlier observations are consistent with the current study's findings. Latha *et al.* (2011) also reported similar improvements, further validating the efficacy of plant-based antiviral treatments in enhancing cocoon quality.

Influence of Plant Extracts on Disease Incidence (%)

The administration of medicinal plant extracts had a significant impact on reducing the incidence of Grasserie disease in the $FC_1 \times FC_2$ bivoltine silkworm hybrid. Compared to the control, all treatment groups exhibited a substantial decrease in disease incidence, demonstrating the antiviral efficacy of the extracts. The lowest disease incidence was recorded in the distilled water control (T_5), followed closely by the *Aloe vera* + *Bm*NPV treatment (T_1) with 23.6%. The *Phyllanthus niruri* + *Bm*NPV group (T_2) showed a moderate disease incidence of 56.9%, while the *Andrographis paniculata* + *Bm*NPV group (T_3) recorded a higher incidence of 75.2%. The highest disease incidence was observed in the *Bm*NPV-only treatment (T_4), with 78.4% (Table 1; Figure 1).

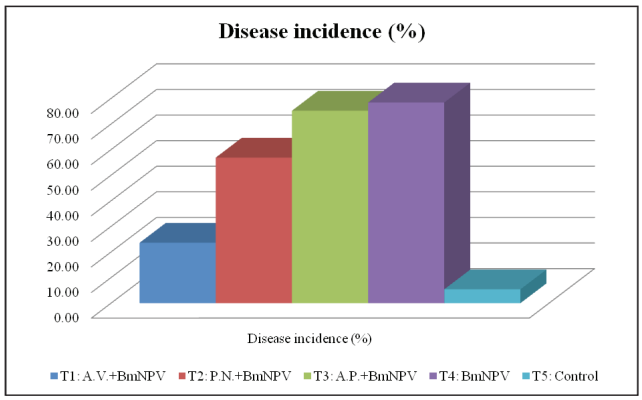

Figure 1: Disease incidence (%) during the rearing of $FC_1 \times FC_2$ bivoltine double hybrid after administration of T_1 to T_5 treatments

These results indicate that *Aloe vera* was particularly effective in suppressing Grasserie disease, significantly reducing the infection rate compared to other plant extracts and untreated *Bm*NPV-infected larvae. The varying degrees of antiviral activity among the plant extracts highlight their potential role in integrated disease management strategies for silkworm rearing (Ram, 2000), with *Aloe vera* emerging as the most promising candidate for reducing viral infections and improving silkworm health.

Sridevi (2003) and Manjunath *et al.* (2020) similarly reported that the incidence of diseases such as Grasserie and Flacherie was significantly reduced in silkworm hybrids CSR2×CSR4 and PM×CSR2 when supplemented with medicinal plant extracts, compared to the control groups. These studies reinforce the findings of the present research, further demonstrating that the incorporation of plant-based treatments can effectively lower disease incidence and enhance the overall health and productivity of silkworms across different hybrid strains. The consistent antiviral efficacy of medicinal plants across various silkworm hybrids underscores their potential as sustainable alternatives in sericulture disease management.

Influence of Plant Extracts on Total Haemocytes Count (THC)

The administration of plant extracts significantly influenced the total haemocyte count (THC) in silkworms infected with *Bm*NPV. In the *Bm*NPV-only treatment group (T_4), THC initially increased from 3,194 ml^{-1} to 5,923 ml^{-1} between the first and third days post-treatment. However, a drastic decline was observed from the fourth day onwards, with THC plummeting to 194 ml^{-1} by the sixth day. In contrast, the control group (T_5) exhibited a steady increase in THC from 3,154 ml^{-1} on the first day to 6,058 ml^{-1} by the fifth day, followed by a reduction to 4,320 ml^{-1} during the spinning stage (Table 2; Figure 2).

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 1×10^5 POB ml^{-1}	3151	5047	5864	4867	4592	2961
T_4: *Bm*NPV	3194	5673	5923	3526	549	194
T_5: Control	3154	4157	4985	5927	6058	4320

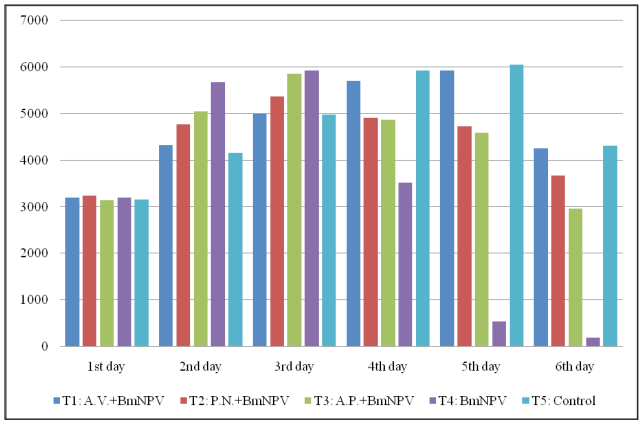

Figure 2: Effect of different medicinal plant treatments on total hemocyte count

The *Aloe vera* + *Bm*NPV treatment (T_1) demonstrated a similar trend to the control group, with THC increasing significantly from 3,205 ml^{-1} to 5,924 ml^{-1} over the first five days post-treatment. By the sixth day, THC decreased to 4,256 ml^{-1}, a value comparable to the control. This suggests that the administration of *Aloe vera* alongside *Bm*NPV inoculation contributed to maintaining haemocyte levels similar to healthy control larvae, likely due to the antiviral properties of the plant extract. Treatments T_2 (*Phyllanthus niruri* + *Bm*NPV) and T_3 (*Andrographis paniculata* + *Bm*NPV) followed the same pattern as T_1, though they ranked third and fourth, respectively, in terms of THC levels.

The findings align with previous studies by Ananda Kumar and Michael (2011) and Nazar *et al.* (2020), who reported that silkworm larvae infected with *Bacillus thuringiensis* exhibited a significant reduction in THC, with Flacherie-infected larvae showing a 15.3% decrease in haemocyte levels compared to healthy larvae. These observations suggest that infected silkworms experience physiological weakening due to reduced feeding capacity, which leads to a gradual decline in THC and overall immunity, making them more susceptible to pathogenic diseases (Balavenkatasubbaiah and Sivaprasad, 2014; Nazar *et al.*, 2020). This underscores the potential role of medicinal plant extracts in bolstering immune responses and enhancing resistance to viral infections in silkworms.

Estimation of Total Soluble Proteins in the Haemolymph

The quantitative estimation of total soluble proteins in the haemolymph of the bivoltine hybrid silkworms showed a consistent increase from the first day through to the spinning stage, with this trend being observed across all treatment groups. This increase in protein levels with larval age aligns with the findings of Ananda Kumar and Michael (2012), further validating the progressive accumulation of haemolymph proteins during silkworm development.

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

Table 3: Impact of different medicinal plant treatments on hemolymph protein levels (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 1×10^5 POB ml^{-1}	32.0	43.7	55.1	63.4	69.9	71.4
T_4: *Bm*NPV	31.6	33.9	35.7	34.7	15.1	12.3
T_5: Control	32.5	47.8	71.3	80.7	86.4	91.3

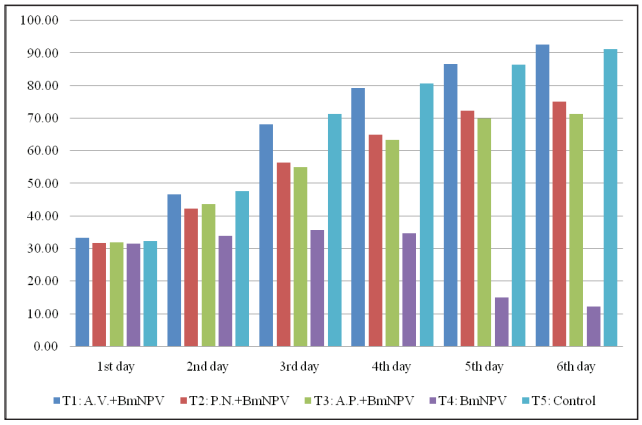

Figure 3: Effect of various medicinal plant treatments on haemolymph protein levels (mg ml^{-1})

These results suggest that the administration of *Aloe vera* extract not only helped maintain higher protein levels despite *Bm*NPV infection but also closely paralleled the protein levels of the healthy control group. The notable decline in protein concentration in the *Bm*NPV-only group highlights the detrimental impact of the virus on silkworm physiology, while the protective role of *Aloe vera* and other medicinal plant extracts in enhancing protein synthesis and immune responses is evident.

Conclusion

Among *Aloe vera*, *Phyllanthus niruri* and *Andrographis paniculata* medicinal plants *Aloe vera* has shown more antiviral activity to suppress the Grasserie disease in silkworm and enhanced the various cocoon parameters. All the medicinal plants extracts have shown positive effects on THC and total protein in haemolymph of mulberry silkworm. Hence, *Aloe vera* powder can also be added in bed disinfectants for the management of viral diseases of silkworm.

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