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SRI-VIPRA

Project Report of 2025: SVP-2507

"Influence of different plant species on life history parameters of *Spodoptera frugiperda* and its salivary enzymes"

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SRIVIPRA PROJECT 2025

Title: Influence of different plant species on life history parameters of *Spodoptera frugiperda* and its salivary enzymes

Name of Mentor: Dr. Vartika Mathur **Name of Department:** Zoology **Designation:** Professor	

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Signature of Mentor

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2507 titled "Influence of different plant species on life history parameters of *Spodoptera frugiperda* and its salivary enzymes". The participants have carried out the research project work under my guidance and supervision from 1st July 2025 to 30th September 2025. The work carried out is original and was carried out in an offline mode.

Signature of Mentor

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1. Introduction

Spodoptera frugiperda (fall armyworm) is a highly polyphagous lepidopteran pest capable of feeding on more than 350 plant species across diverse families (Rwomushana, 2019). Its ability to utilize such a wide host range is closely linked to its adaptive feeding strategies, digestive physiology, and secretion of salivary enzymes that mediate early plant-insect interactions (Cai et al., 2025). Salivary secretions not only facilitate digestion but also play a pivotal role in modulating plant defense responses, thereby directly influencing insect performance and life history traits such as growth rate, survival, development, and fecundity (Scherr, 2025). Previous studies have chiefly focused on its major agroeconomic hosts such as maize, rice, sorghum, cotton, and certain legumes, examining how these affect pest development and physiology (Nurkomar et al., 2023). For instance, studies have confirmed that *S. frugiperda* larvae raised on maize or other cereals exhibit rapid growth, high pupal weights, and maximum reproductive potential, while alternative hosts like sweet potato or cowpea led to reduced fitness, delayed development, and lower fecundity (Costa et al., 2020; Gong et al., 2023). These differences are often attributed to host-specific nutritional profiles, defensive compounds, and variations in plant secondary metabolites.

Despite its importance, there is a significant gap in the literature concerning direct, comparative evaluations of hosts from more diverse families (particularly those outside cereals and legumes) that are accessible and locally important. Studies examining crops such as castor (Euphorbiaceae), mulberry (Moraceae), rice (Poaceae), banana (Musaceae), and papaya (Caricaceae) as experimental hosts are especially rare, with most existing reports either focusing on a single host at a time or pooling data from a limited set of hosts. Importantly, there has been limited investigation into how these varied hosts affect larval enzyme profiles, especially salivary enzymes that initiate plant tissue breakdown and may modulate plant defense responses, despite known differences in enzymes like amylases and proteases among insects feeding on diverse plant types.

In the present study, these five plants were selected because they represent distinct families commonly grown and available on college campuses, differing markedly in physical and chemical defenses, nutritional quality, and ecological context. By systematically comparing *S. frugiperda* performance and its salivary enzyme activity on castor, mulberry, rice, banana and papaya, this work addresses two critical gaps: (i) the lack of multi-family, side-by-side host comparisons relevant to local agroecosystems, and (ii) insufficient data linking host-driven variation in insect life history parameters directly to shifts in digestive physiology and potential pest resilience mechanisms. This approach thus expands our understanding beyond the typically studied cereal-legume continuum, providing unique insight into how host diversity shapes *S.*

frugiperda adaptation and highlighting possible avenues for integrated pest management based on host resistance traits.

2. OBJECTIVES

The following were the objectives of our study:

1. To evaluate the development and feeding preference of *Spodoptera frugiperda* on five different host plants
2. To analyze the changes in antioxidants and salivary enzyme activities in *S. frugiperda* after feeding on different plant species
3. To identify the salivary microbiome in *S. frugiperda* after feeding on different plant species

3. METHODOLOGY

3.1 Plant

Plants of different families were chosen, which were commonly present at Sri Venkateswara College (28.5894° N, 77.1681° E), New Delhi, India (Table 1).

Table 1: List of plants and their families chosen for experiments

Sr. No.	Plant	Family
1.	Castor (*Ricinus communis*)	Euphorbiaceae
2.	Mulberry (*Morus spp.*)	Moraceae
3.	Rice (*Oryza sativa*)	Poaceae
4.	Banana (*Musa acuminata*)	Musaceae
5.	Papaya (*Carica papaya*)	Caricaceae

3.2 Insect Culture Conditions and Treatment

S. frugiperda larvae were obtained from the laboratory-reared culture on artificial diet containing Bengal gram flour as per ICAR-IARI, New Delhi. Larvae were reared at 27 ± 1 °C temperature, 60-70 % relative humidity and 14 L:10 D photoperiod. The pupae were sterilized using 1.6 % sodium hypochlorite solution. The moths were given 10% honey solution supplemented with multivitamins throughout the egg-laying period. The larvae were periodically mixed with a field-collected population of *S. frugiperda*. First 12 instar larvae were given leaves from each treatment. Feeding preference assay and biochemical assays were performed on 4th instar larvae.

3.3 Larval Mortality Rate and Biomass

The leaves from each treatment were fed to larvae of *S. frugiperda* from first instar larvae till 6th instar larvae. Growth stage and mortality of each larva were recorded daily and the % mortality rate of larvae was recorded using Selin-Rani et al. (2016).

3.4 Insect nutritional indices

The leaf from each set was collected and weighed immediately. The leaf was placed in a 90 mm Petri plate. Freshly molted 4th instar *S. frugiperda* that were satiated with water for four hours before the experiment, and were placed in a 6-well plate with 12 replicates per set. The larval weight gain, unconsumed leaf, and insect faeces were weighed after three days. Nutritional indices, including Relative Consumption Rate (RCR), Relative Growth Rate (RGR), Approximate Digestibility (AD), Efficiency of Conversion of Ingested food (ECI), and Efficiency of Conversion of Digested food (ECD) were determined according to Waldbauer (1968).

3.5 Feeding Preference Assay

The feeding preference assay was performed by using the methodology of Mathur et al. (2011). A glass Petri dish (20 cm diameter) with moist filter paper was used for the feeding preference assay. Leaves from different plants were excised, outlined on graph paper, and arranged equidistantly in the Petri dish. One 4th instar larva of *S. frugiperda* was introduced at the centre of the dish and allowed to move and feed freely on leaves between each treatment. After 24 h, the larvae were removed, and the damaged portion of the leaves was traced on graph paper. Leaf area consumed was quantified by comparing the damaged area to the original leaf outline. The assay was replicated 5 times.

3.6 Secondary Metabolites

Haemolymph was extracted from each larva (n=12) from the different treatments by incising the proleg with micro scissors under sterile conditions. The extraction for the antioxidant was performed using Kaur et al. (2021). The collected haemolymph was then mixed with 1 mL Phosphate-buffered saline (PBS) and centrifuged at 8,000 rpm for 20 min at 4 °C. The supernatant was collected and catalase (CAT), peroxidase (POD), and Ascorbate Peroxidase (APX) activity were analysed.

3.7 Salivary enzymes

Salivary glands of 4th instar larvae of *S. frugiperda* were dissected under a stereomicroscope in ice-cold saline buffer as per Zhang et al. (2025). Using fine forceps and micro-scissors, head was carefully removed to expose the salivary glands. After that, paired salivary glands were gently pulled out, which are located near the head-thorax junction. Dissected glands were immediately placed into a pre-chilled microcentrifuge tube containing cold extraction buffer (phosphate-buffered saline) and then centrifuged at 12,000 rpm at 4°C for 12 min. to obtain the supernatant containing soluble salivary proteins. The supernatant was immediately stored at -80°C for further analysis of α-amylase and serine protease inhibitors (PI) activity.

3.8 Endosymbiont density and morphological diversity

CFU of insect's endosymbionts was determined using a combined and modified methodology based on the protocols described by Koga et al. (2009), Grobler (2019) and Sivaramakrishnan and Razia (2021). Fourth instar larvae from each treatment were sterilized with 70 % ethanol before dissection. The gut was removed and placed in a sterile petri dish. The dissection was done from last abdominal segment to the first thoracic segment to expose the gut. Three midguts from all the experimental sets (n=3) were pooled and placed in single 1.5 mL sterile microcentrifuge tube which contained 1 mL of DDW. Thereafter, 100 µL of the supernatant was poured into NAM, R2A and PDA medium. Culture plates were examined for the number of colony-forming units (CFU) and morphologically identified on the basis of form, margin, elevation, colour and texture.

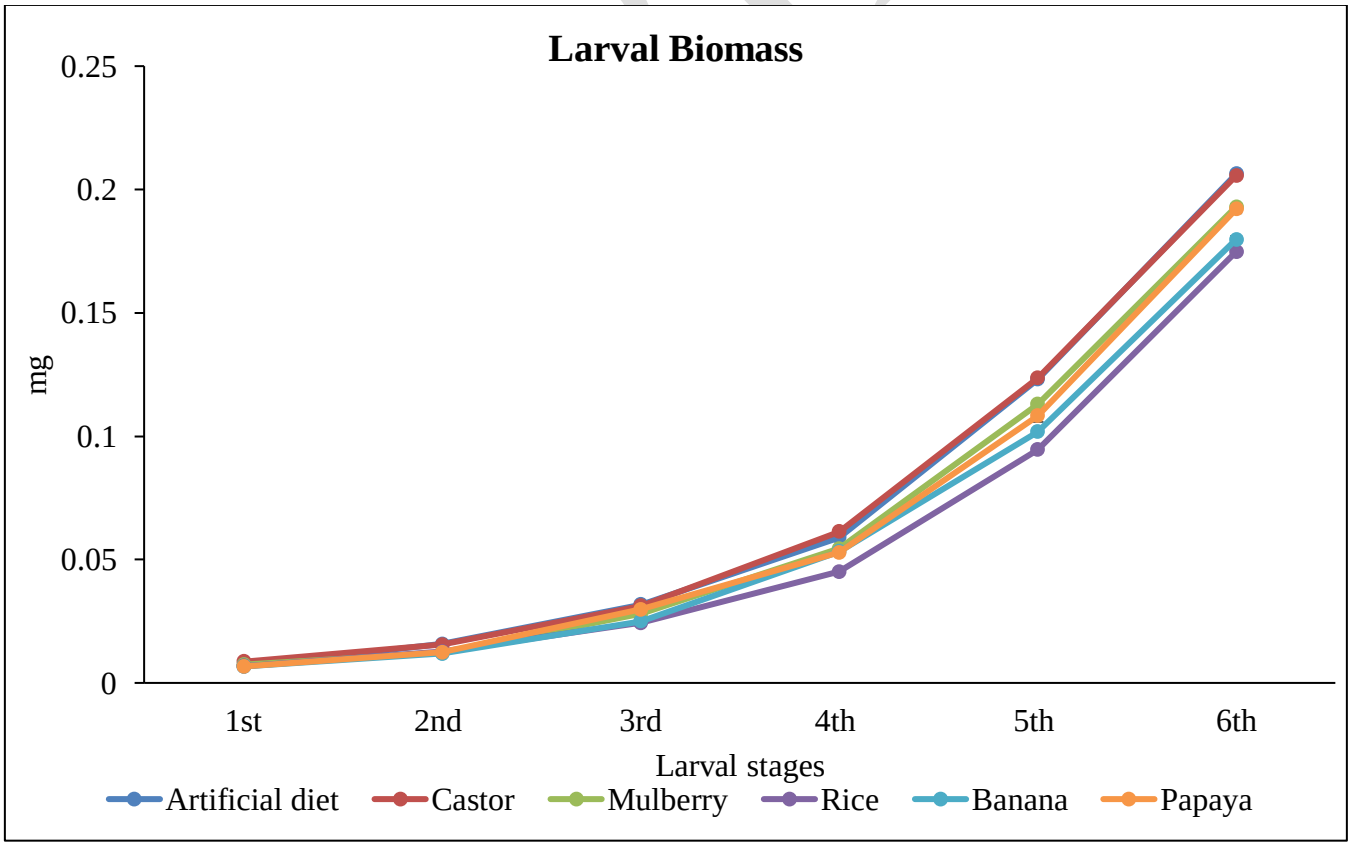
Result

1. Larval Mortality Rate and Biomass

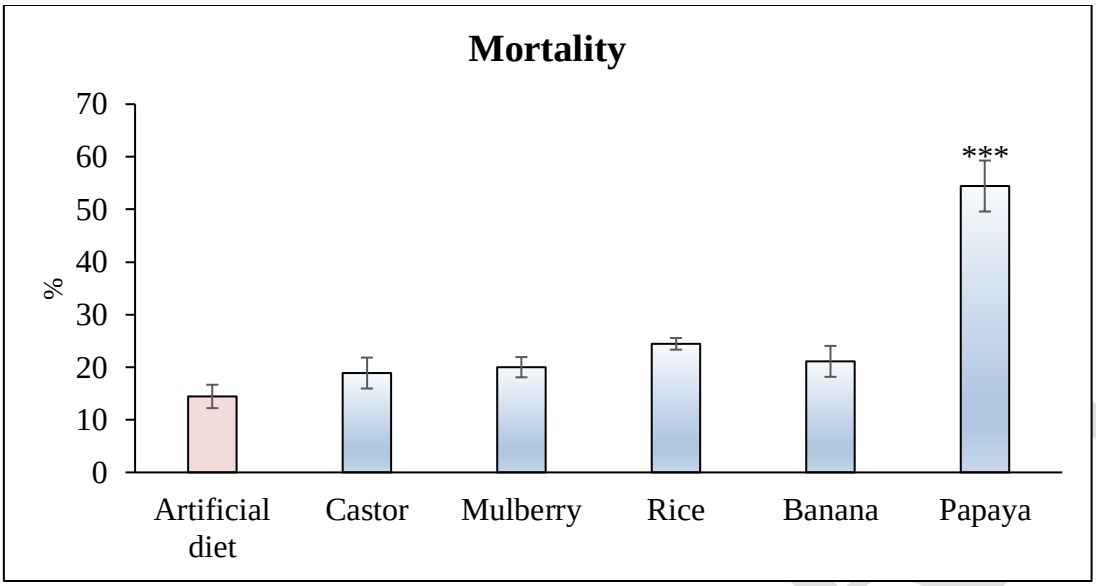
No significant difference was observed in 1st (p>0.05) larval stage duration. However, duration of 2nd larval stage of banana (p<0.001) and 3rd larval stage of banana and papaya (p<0.001) and 4th larval stage of banana and papaya (p<0.001) was significantly increased. Similarly, duration of 5th larval stages of mulberry, rice, banana, papaya (p<0.001) and 6th larval stages of mulberry, banana and papaya showed significant difference as compared to artificial diet.

Further weight of 1st larval stage of castor (p<0.001) showed significant increased. In contrast, 2nd larval stage of mulberry, rice, banana and papaya (p<0.001), 3rd larval stage of banana and rice (p<0.001) and 4th larval stage of rice, banana and papaya (p<0.001) was significantly decreased. Similarly, weight of 5th larval stage of mulberry, rice, banana and papaya (p<0.001) and 6th larval stages of mulberry, rice, banana and papaya (p<0.001) was significantly decreased (Figure 1a).

Additionally, their mortality was notably higher in insects fed on papaya (p<0.001) compared to other plants (Figure 1b).



(a)



(b)

Figure 1: *S. frugiperda* fed on different host plants. a) Larval biomass *of* insect, and b) mortality of insect fed on different hosts. (***), (**) and (*) indicate significant differences at p<0.001, p<0.005 and p<0.05 respectively

2. Insect nutritional indices

CI by the 4th larval stage of insect was significantly reduced in castor, mulberry and papaya (p<0.001). In contrast, RGR (p<0.001), AD (p<0.001), ECI (p<0.001) and ECD (p<0.001) was significantly difference in insect feed on all plant host as compared to artificial diet.

3. Feeding Preference Assay

A significant increase in the feeding preference of insect fed on castor (p<0.001).

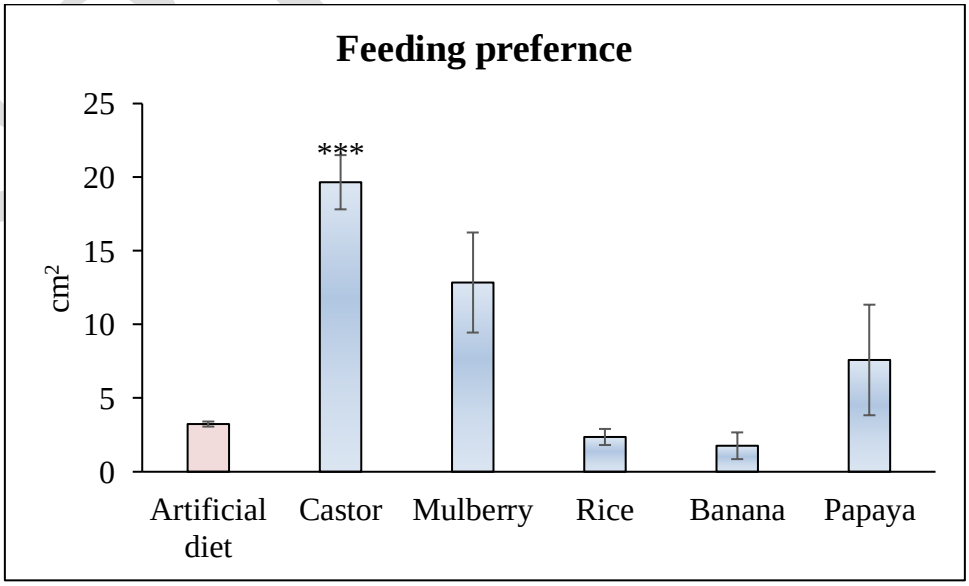
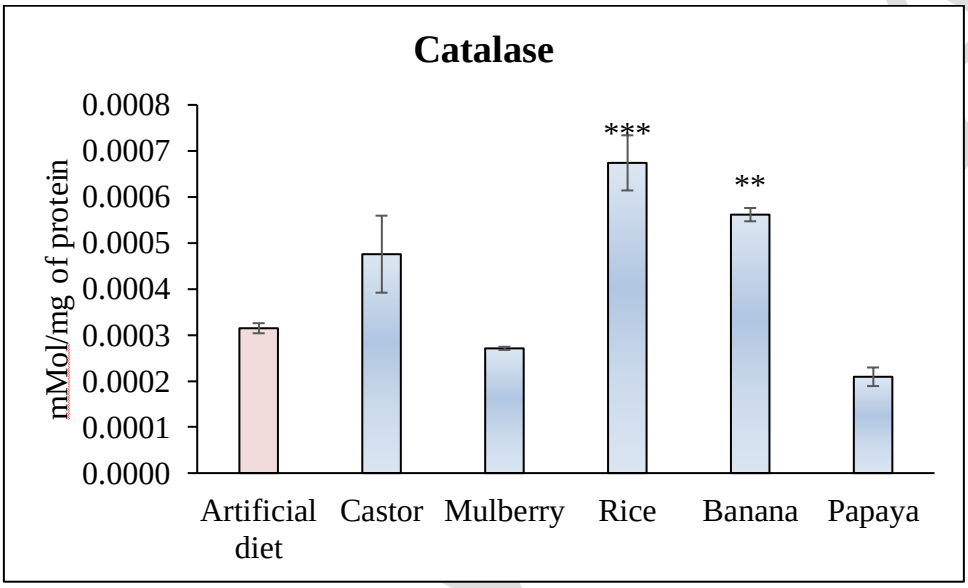


Figure 2: Feeding preference of *S. frugiperda* larvae: feeding preference of larvae on different host plant leaves. (***), (**) and (*) indicate significant differences at p<0.001, p<0.005 and p<0.05 respectively

4. Secondary Metabolites

Catalase activity showed significantly increased in rice and banana (p<0.001; Figure 3a). Similarly, POD activity was significantly decrease in banana (p<0.001; Figure 3b). In contrast, APX activity showed significant increased in all plant hosts (p<0.001; Figure 3c).



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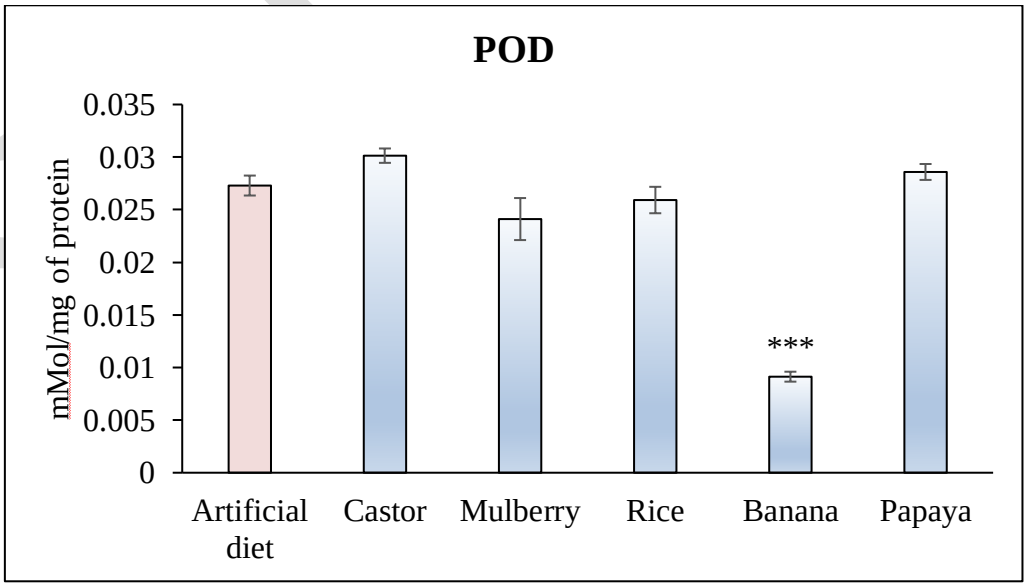

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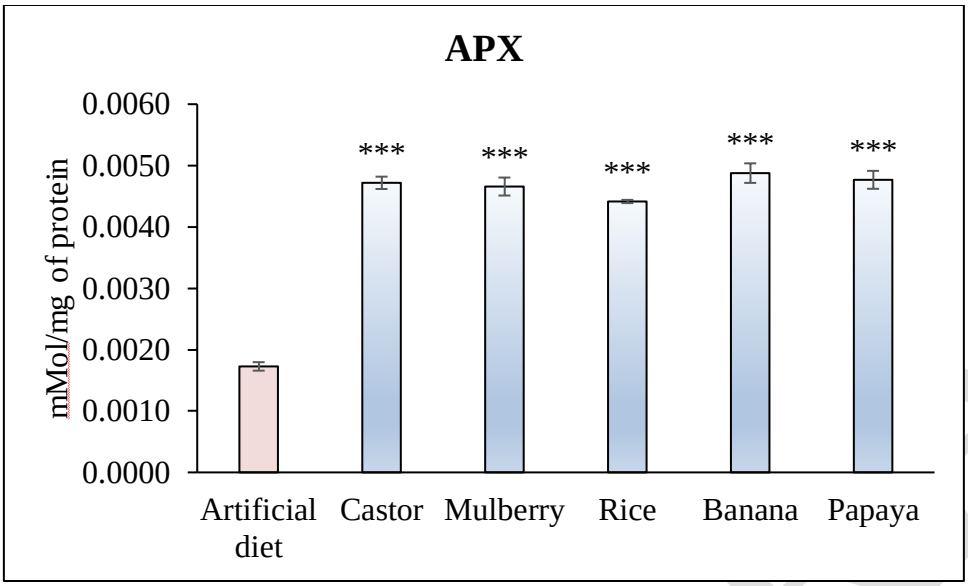
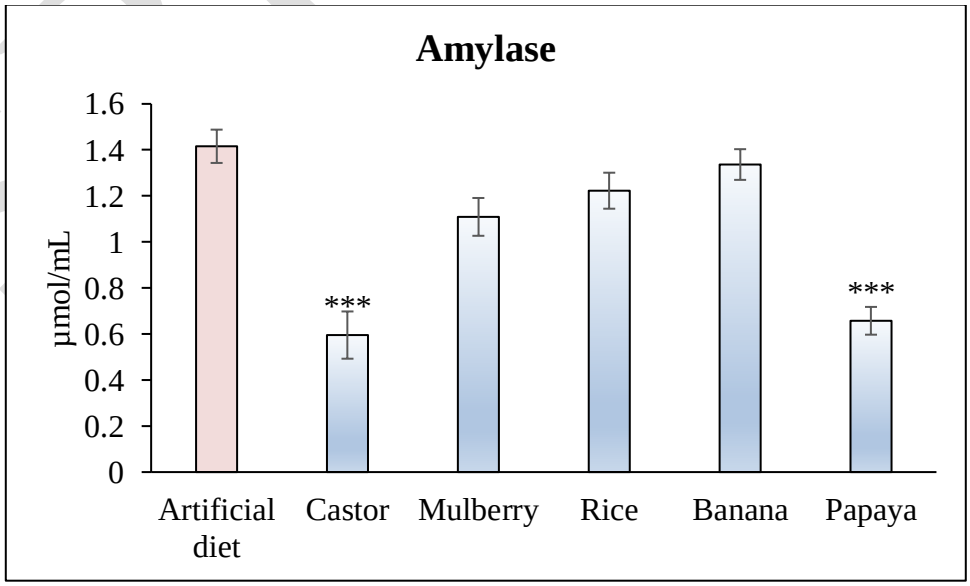


(c)

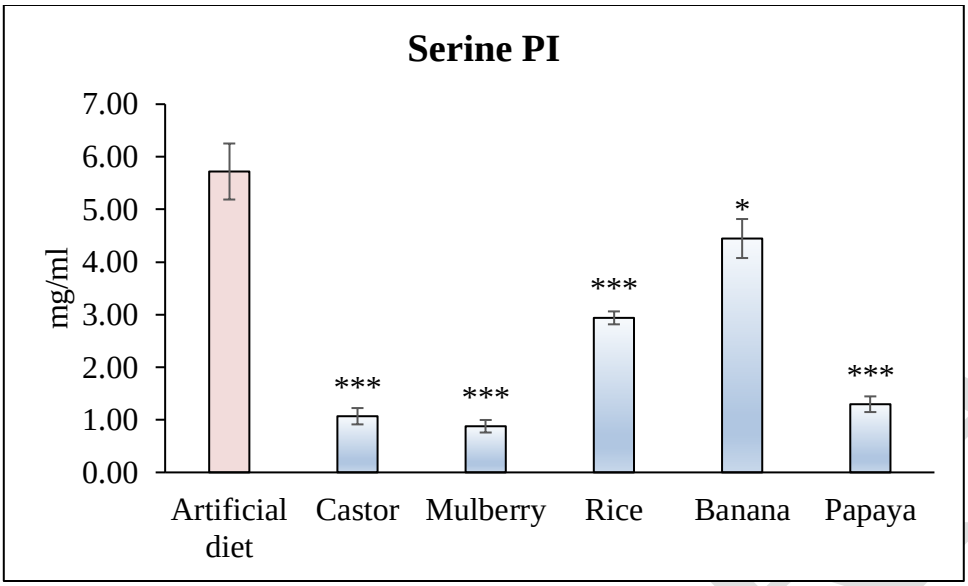
Figure 3: Antioxidant enzyme activities in larvae: a) catalase, b) peroxidase, and c) ascorbate peroxidase activity in larvae exposed to different host plants. (***), (**) and (*) indicate significant differences at $p<0.001$, $p<0.005$ and $p<0.05$ respectively

5. Salivary enzymes

Amylase activity was significantly reduced in castor and banana ($p<0.001$; Figure 4a). Similarly, Serine protease inhibitor was significantly reduced in all plant host ($p<0.05$; Figure 4b) as compared to the artificial diet.



(a)

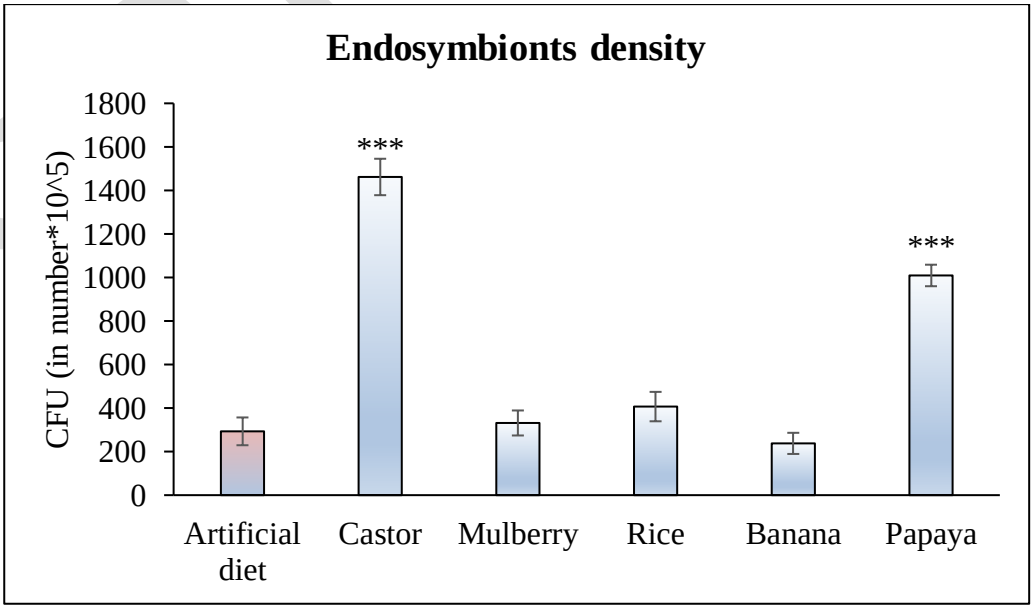


(b)

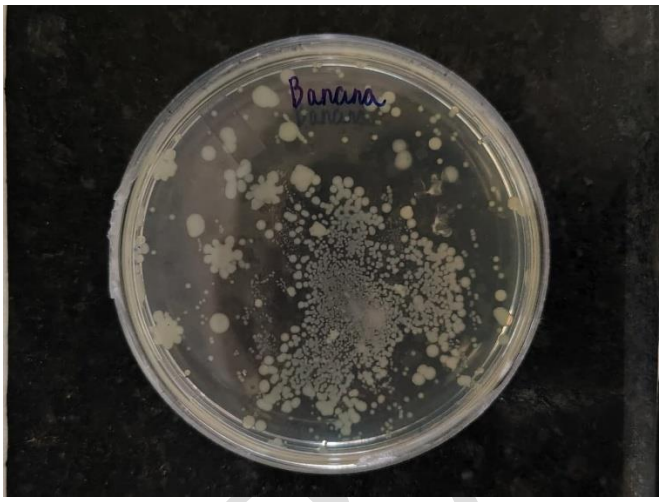
Figure 4: Salivary enzyme activities in larvae: a) amylase, b) serine protease inhibitors activity in larvae exposed to different host plants. (***), (**) and (*) indicate significant differences at p<0.001, p<0.005 and p<0.05 respectively

6. Endosymbiont density and morphological diversity

The CFU count of endosymbionts was significantly increased in insects fed on castor and papaya (p<0.001; Figure 5a). Similarly, based on morphological observations, endophytic diversity was also reduced in insects fed on castor, rice and papaya (Table 2).



(a)



(b) **(c)**

Figure 5: Microbial CFU of endosymbionts in insect: a) Endosymbionts density, and b-c) endosymbionts growing on NAM. (***), (**) and (*) indicate significant differences at $p<0.001$, $p<0.005$ and $p<0.05$ respectively

Table 2: Morphological diversity of endosymbionts isolated from the insect fed on different plant hosts

S. No	No. of colonies	Shape	Elevation	Margins	Texture	olor
			Artificial diet			
1.	85	Circular	Raised	Entire	Mucoid	Pale yellow
2.	988	Circular	Raised	Entire	Mucoid	White
3.	7	Irregular	Flat	Undulate	Dry	White
4.	501	Punchiform	Raised	Entire	Mucoid	Pale yellow
5.	31	Rhizoid	Raised	Filliform	Mucoid	Pale yellow
6.	147	Filamentous	Flat	Filliform	Dry	White
			Castor			
1.	31	Circular	Raised	Entire	Mucoid	Pale yellow
2.	3142	Circular	Raised	Entire	Mucoid	Yellow
3.	4212	Irregular	Crateriforms	Entire	Mucoid	White
4.	1386	Irregular	Raised	Entire	Mucoid	Yellow
			Mulberry			
1.	85	Irregular	Flat	Undulate	Mucoid	White
2.	1106	Circular	Flat	Entire	Mucoid	White
3.	10	Irregular	Flat	Filliform	Mucoid	White

4.	1	Irregular	Flat	Lobate	Mucoid	White
5.	256	Circular	Flat	Entire	Mucoid	Yellow
6.	24	Irregular	Flat	Undulate	Mucoid	Yellow
7.	6	Irregular	Flat	Lobate	Mucoid	Yellow
8.	490	Circular	Raised	Undulate	Mucoid	Yellow
9.	12	Irregular	Raised	Lobate	Mucoid	Yellow
			Rice			
1.	769	Circular	Raised	Entire	Mucoid	Pale yellow
2.	422	Circular	Raised	Entire	Mucoid	Off white
3.	1248	Irregular	Raised	Entire	Mucoid	Off white
4.	3	Irregular	Raised	Undulate	Mucoid	Off white
			Banana			
1.	11	Irregular	Flat	Undulate	Mucoid	White
2.	1160	Circular	Flat	Entire	Mucoid	White
3.	1	Rhizoid	Flat	Filliform	Mucoid	White
4.	5	Irregular	Flat	Undulate	Mucoid	Yellow
5.	90	Irregular	Flat	Entire	Mucoid	Yellow
6.	160	Circular	Flat	Entire	Mucoid	Yellow
			Papaya			
1.	2227	Circular	Raised	Entire	Mucoid	Pale yellow
2.	3829	Circular	Raised	Entire	Mucoid	White

Discussion:

The study's findings make it abundantly clear that the host plant has a significant impact on the insect's developmental biology, nutritional physiology, antioxidant responses, and gut microbial dynamics. While there was no discernible difference in the duration of the first larval instar, but from the 2nd larval instar onwards, feeding on banana and papaya caused a noticeable delay in development. This extension of the larval period may be explained by the presence of defensive secondary metabolites in these hosts, which slow down growth and lengthen the feeding period, or by an adaptive reaction to poor nutritional quality (Maharjan et al., 2023). In addition, when insects were raised on mulberry, rice, banana, and papaya instead of the artificial diet, the larval biomass in later stages was consistently lower. This weight loss suggests ineffective nutrient absorption, most likely due to structural defenses or limitations imposed by plant chemistry. Interestingly, the highest mortality rate was observed in larvae that fed on papaya, supporting the idea that papaya offers the most unfavorable conditions for insect development, possibly because it contains more defensive compounds or fewer nutrients (Aslan et al., 2025).

The biochemical reactions also highlight the larvae's attempts to manage oxidative stress brought on by consuming various plants. While decreased peroxidase activity in banana-fed larvae suggests compromised defensive responses that may make them more vulnerable to oxidative damage, elevated catalase activity in insects fed on rice and banana indicates an upregulation of detoxification pathways to combat reactive oxygen species. APX was consistently elevated in all plant-fed larvae, indicating that it functioned as a compensatory mechanism in response to oxidative stress brought on by substances derived from plants (Vellau et al., 2013). Additionally, strong host effects were revealed by digestive enzyme activities. The overall suppression of serine protease inhibitor activity in all plant-fed larvae suggests weakened protein metabolism, whereas the decrease in amylase activity in larvae fed castor and bananas suggests impaired digestion of carbohydrates. Plant enzyme inhibitors or secondary metabolites that directly disrupt digestive physiology are responsible for these reductions (Karlsson Green, 2021).

Lastly, modifications to the dynamics of gut microbes offer more information about host-mediated impacts. Larvae fed castor and papaya showed a marked increase in colony-forming units, which indicates that microbes are proliferating in response to dietary stress. However, a disturbance in gut homeostasis is reflected in the concurrent decrease in microbial diversity in larvae fed papaya, rice, and castor. As effective digestion and nutrient absorption depend on microbial diversity, this dysbiosis probably makes the detrimental effects on larval performance worse, which is especially noticeable in the high papaya mortality rate (Yang et al., 2022).

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