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Exploring the influence of *Spinacia oleracea* extract on silkworm development and silk characteristics: A comprehensive study

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Abstract
The impact of an ethanolic extract obtained from *Spinacia oleracea* on *Bombyx mori L.* silkworm larvae was investigated, revealing notable findings across various parameters. The study involved an assessment of larval weight, total mortality, cocoon weight, shell weight, pupal weight, shell ratio, filament length, filament weight, denier, and the number of breakages. Upon analysis, results reveal a concentration-dependent relationship between the extract and multiple developmental parameters. Higher concentrations correlate with increased larval weights, decreased mortality rates, and enhanced cocoon characteristics such as weight, shell attributes, and silk quality. These findings suggest the extract's potential to positively influence sericulture and silk industry practices.

Keywords: *Spinacia oleracea*, mulberry, susceptibility, compounds

Introduction

Sericulture, the art and science of cultivating silkworms for the production of silk, stands as one of the oldest and most revered agro-based industries globally (Biswas *et al*., 2016) [2]. This millennia-old practice intertwines human civilization with the natural world, encapsulating a harmonious synergy between traditional knowledge and modern advancements. The exquisite and luxurious silk fibre, renowned for its softness, sheen, and durability, has not only adorned civilizations but also fueled economies, trade routes, and cultural exchanges across continents. The exploration of natural compounds and their potential impact on the growth and development of organisms has long intrigued researchers across various scientific disciplines (Harvey, 2008) [4]. Among these compounds, the ethanolic extract derived from *Spinacia oleracea*, commonly known as spinach, emerges as a subject of interest due to its rich nutritional profile and purported medicinal properties (Miano, 2016; Lasya, 2022) [11, 3]. In particular, investigating the effects of this extract on the larvae of *Bombyx mori* L., the silkworm renowned for its silk production, presents an intriguing avenue for scientific inquiry (Selv *et al*., 2014) [8]. Silkworms, integral to the ancient art of sericulture, offer a unique opportunity to study the physiological responses and potential benefits of natural extracts. Their well-defined developmental stages, susceptibility to external influences, and economic importance in silk production make them an ideal model organism for such investigations. The utilization of *Spinacia oleracea* extract, renowned for its diverse bioactive compounds, introduces a novel angle to understand its potential impact on the growth, physiology, and silk production of *Bombyx mori* L. larvae.

The present study endeavours to delve into the effects of *Spinacia oleracea* ethanolic extract on various parameters of *Bombyx mori* L. larvae. By analysing factors such as larval weight, cocoon characteristics, pupal development, and silk quality, this study aims to elucidate the potential influence of the extract on the overall growth and silk-producing capabilities of these larvae. Through meticulous observation and quantitative analysis, this research seeks to contribute to our understanding of the potential applications of *Spinacia oleracea* extract in enhancing the productivity and quality of silk, thereby potentially offering insights into innovative approaches in sericulture and related fields. The present investigation not only bridges the realms of natural products and sericulture but also underscores the significance of exploring eco-friendly and sustainable methods to augment silk production. The findings from this study hold the promise of not only expanding our knowledge of the intricate interplay between natural extracts and organismal development but also potentially paving the way for practical applications in silk production, benefiting both the scientific community and the sericulture industry.

Material and Methods

Rearing of *Bombyx mori* L.

The second instar's larvae of *Bombyx mori* were procured from the chowki rearing centre, Umarkhed, District Yavatmal, during October 2023 brought in the laboratory, second instar to fourth instars larvae were reared on fresh Mulberry leaves, fifth instars larvae were taken for experimental study, and they were grouped into five batches. The Rearing was carried out and incubated as per Hiware (2001) [5].

Collection of plant material

Spinacia oleracea seeds were acquired from a market in Pusad, District Yavatmal. The seeds were thoroughly washed with distilled water to remove impurities and debris. Post-washing, the seeds were air-dried in shaded conditions to avoid direct sunlight exposure. Once dried, the seeds were ground into a fine powder using an electric grinder. The powdered seeds were carefully stored in airtight polythene bags to preserve freshness and protect against moisture and pests.

Preparation and treatment of crude plant extract

The 40-gram powder obtained from *Spinacia oleracea* seeds was subjected to extraction using 400 ml of Ethanol via a Soxhlet extraction apparatus following the methodology outlined by Lolge *et al*. (2016) [10]. Upon completion of the extraction process, the resultant extract was exposed to air for solvent evaporation. The remaining extract was stored as a stock solution in a refrigerator until further use. For larval treatment, three distinct concentrations were prepared by dissolving 1 gram of the crude extract in 100 ml of distilled water to achieve concentrations of 1%, 2%, and 3%. These concentrations were attained by adjusting the volume with the requisite amount of distilled water. Subsequently, the solutions were separately sprayed onto air-dried mulberry leaves using a sprayer, which were then provided as food to the larvae under study. Throughout the study, various parameters including larval weight, total mortality, cocoon weight, shell weight, pupal weight, and shell ratio were meticulously measured to assess the effects of the different concentrations on the larvae. This methodological approach was aimed at investigating the impact of varied concentrations of *Spinacia oleracea* seed extract on the measured parameters within the larval study.

These formulas seem to be used for calculating specific parameters related to cocoons and silk reeling. Here they are presented with a clearer structure:

$$\text{Cocoon shell weight} = \frac{Weight\ of\ shell}{TOtal\ Number\ of\ shell}$$

$$\text{Cocoon shell Ratio} = \frac{Weight\ of\ shell}{Weight\ of\ cocoon} \times 100$$

$$\text{Denier} = \frac{Weight\ of\ raw\ silk\ reeled\ (g)}{Weight\ of\ raw\ silk\ reeled\ (m)} \times 9000$$

These formulas seem to be essential for assessing the characteristics and quality of the silk obtained from cocoons, providing insights into the shell weight, shell ratio, and denier of the silk produced (Avhad and Hiware, 2016) [1].

Results and Discussion

The larval weights observed across different concentrations of *Spinacia oleracea* extract were as follows: in the control group, the average larval weight measured 2.650 grams. Meanwhile, larvae subjected to the 1% concentration exhibited an increased average weight of 3.401 grams. Further concentration increments corresponded to higher larval weights, with the 2% concentration resulting in an average weight of 4.084 grams, and the 3% concentration showing the highest average larval weight recorded at 4.501 grams. These findings suggest that *Spinacia oleracea* extract supplementation positively influences the growth and development of *Bombyx mori* larvae. Additionally, similar results were observed in a separate study where larval weight increased significantly in response to a 25% concentration of *Spinacia oleracea* extract (Selvi *et al*., 2014) [8].

The total mortality rates observed among larvae subjected to varying concentrations of *Spinacia oleracea* extract were as follows: within the control group, the total mortality recorded was 5 individuals out of 50. In contrast, the larvae treated with a 1% concentration exhibited a decreased mortality rate of 3 individuals out of 50. Further reductions in mortality were evident with increasing concentrations, as the 2% concentration displayed a mortality rate of 1. Strikingly, at the highest concentration of 3%, no mortality was observed among the treated larvae.

The observed decline in total mortality rates as the concentration of *Spinacia oleracea* extract increased suggests a potential correlation between the extract and reduced larval mortality. The absence of mortality in the group treated with the highest concentration indicates a substantial protective effect or potential insecticidal properties of the extract against the studied larvae. These findings hint at the possibility of the extract containing bioactive compounds that can influence larval survival rates.

The cocoon weights obtained from the various concentrations of *Spinacia oleracea* extract were as follows: within the control group, the average cocoon weight measured 1.555 grams. In comparison, the cocoon weights increased with the application of the extract concentrations: the 1% concentration exhibited an average weight of 1.812 grams, the 2% concentration resulted in an average weight of 2.245 grams, and the 3% concentration showed the highest average cocoon weight recorded at 2.599 grams.

The ascending trend in cocoon weights corresponding to increasing concentrations of *Spinacia oleracea* extract suggests a potential dose-dependent effect on silk production or cocoon development. This increase in cocoon weights indicates a stimulatory impact of the extract on cocoon formation or silk gland activity within the larvae. The observed trend might be attributed to bioactive compounds present in the extract, potentially influencing the silk-spinning process or promoting silk gland development, resulting in larger cocoon sizes. Understanding the specific mechanisms underlying this effect warrants further exploration, delving into the biochemical composition of the extract and its interactions with the larvae. These findings indicate the potential utility of *Spinacia oleracea* extract in enhancing silk production or cocoon quality, hinting at its possible applications in sericulture or silk industry-related practices. Additionally, it is noteworthy to mention a similar result observed in a previous study where the extract of *Tribulus Terrestris* exhibited increased cocoon weight. This parallel finding suggests a broader applicability of plant extracts in enhancing cocoon development and silk production in Bombyx mori (Murugan, 1998) [6].

The shell weights recorded from the various concentrations of *Spinacia oleracea* extract were as follows: within the control group, the average shell weight measured 0.331 grams. In contrast, the shell weights displayed an increasing trend with the application of the extract concentrations. Specifically, the 1% concentration showed a slight increase with an average weight of 0.340 grams. The trend continued with the 2% concentration, resulting in an average shell weight of 0.510 grams, and the highest average shell weight was observed in the 3% concentration group at 0.610 grams.

The escalating trend in shell weights corresponding to increasing concentrations of *Spinacia oleracea* extract suggests a potential influence on cocoon shell development or composition. This incremental effect on shell weight could imply a stimulating impact of the extract on silk gland activity or silk protein synthesis within the larvae, resulting in denser or heavier shells. The observed trend might be linked to specific bioactive compounds present in the extract, potentially enhancing silk quality or affecting the

composition of the cocoon shell. Exploring the biochemical constituents of the extract and their mechanisms in influencing silk protein synthesis or cocoon formation would shed light on the underlying processes. These findings signify the extract's potential role in enhancing cocoon shell weight, indicating its prospective applications in sericulture or silk industry-related practices. This aligns with previous research by Bhat *et al*., (2022) [7], where the oral administration of Zea mays flour to *Bombyx mori* L. significantly showed an upsurge in almost all biological and commercial traits. This suggests a broader potential for natural additives in influencing the traits of silk-producing insects, providing avenues for further investigation and application in sericulture practices.

The pupal weights obtained from varying concentrations of *Spinacia oleracea* extract were as follows: within the control group, the average pupal weight measured 1.232 grams. As the concentrations of the extract increased, there was a noticeable rise in pupal weights. Specifically, the 1% concentration exhibited an increased average weight of 1.455 grams. The trend continued with the 2% concentration, resulting in an average pupal weight of 1.701 grams, and the highest average pupal weight was observed in the 3% concentration group at 1.939 grams.

The ascending trend in pupal weights corresponding to increasing concentrations of *Spinacia oleracea* extract suggests a potential dose-dependent effect on pupal development. This incremental increase in pupal weights may indicate a stimulating impact of the extract on pupation or metamorphosis, potentially influencing the pupal growth rate or nutrient utilization by the larvae during the pupal stage. The observed trend might be linked to specific bioactive compounds present in the extract, potentially enhancing nutrient assimilation or regulating developmental processes leading to increased pupal size.

The shell ratios, observed in the cocoons of *Bombyx mori* subjected to various concentrations of *Spinacia oleracea* extract were as follows: within the control group, the average shell ratio measured 16.286%. Upon application of the 1% concentration, the shell ratio increased to an average of 18.764%. Furthermore, the trend continued with the 2% concentration, resulting in an average shell ratio of 22.717%. The highest average shell ratio was observed in the 3% concentration group, recording an average of 23.471%.

The increasing trend in shell ratios corresponding to escalating concentrations of *Spinacia oleracea* extract suggests a potential dose-dependent effect on cocoon shell composition or structure. This increment in the shell ratio implies a relative increase in the proportion of silk protein or the denseness of the cocoon shells. The extract may be influencing the biosynthesis or deposition of silk proteins within the silkworms, resulting in thicker or more compact cocoon shells. The observed trend may be linked to specific bioactive compounds within the extract, potentially regulating silk protein production or affecting the spinning process, consequently altering the cocoon shell characteristics. Delving into the biochemical constituents of the extract and their influence on silk protein synthesis or cocoon formation could elucidate the mechanisms behind this effect. These findings highlight the extract's potential in influencing cocoon shell ratios

The filament lengths of silk reeled from cocoons, measured in meters, varied across different concentrations of *Spinacia oleracea* extract. Within the control group, the average filament length was recorded at 748 meters. As the concentrations of the extract increased, there was a notable increase in filament length: the 1% concentration exhibited an average length of 757.6 meters. Subsequently, the 2% concentration displayed a further increase to an average of 805.5 meters. The highest average filament length was observed in the 3% concentration group, recording an average of 956 meters.

The escalating trend in filament lengths corresponding to increasing concentrations of *Spinacia oleracea* extract suggests a potential dose-dependent effect on silk quality or filament elongation. This increase in filament length implies an augmentation in the length of individual silk strands reeled from the cocoons. The extract might be influencing the properties of silk proteins or the spinning process, resulting in longer and potentially stronger silk filaments. The observed trend may be attributed to specific bioactive compounds within the extract, potentially impacting silk protein synthesis or the mechanical properties of silk fibres. Exploring the biochemical constituents of the extract and their influence on silk fibre formation could provide insights into the underlying mechanisms. These findings underscore the extract's potential in enhancing filament length.

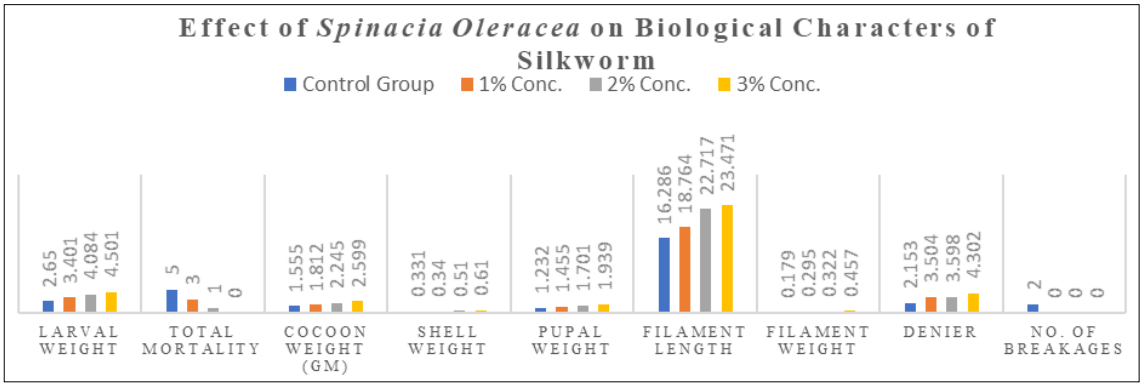
The filament weights of silk, measured in grams, reeled from cocoons varied across different concentrations of *Spinacia oleracea* extract. Within the control group, the average filament weight was recorded at 0.179 grams. As the concentrations of the extract increased, there was a noticeable increase in filament weight: the 1% concentration exhibited an average weight of 0.295 grams. Subsequently, the 2% concentration displayed a further increase to an average of 0.322 grams. The highest average filament weight was observed in the 3% concentration group, recording an average of 0.457 grams.

The ascending trend in filament weights corresponding to increasing concentrations of *Spinacia oleracea* extract suggests a potential dose-dependent effect on silk quality or filament density. This increase in filament weight implies a higher mass of individual silk strands reeled from the cocoons. The extract might be influencing the silk protein composition, spinning process, or silk gland activity, resulting in denser or more substantial silk filaments. The observed trend may be linked to specific bioactive compounds within the extract, potentially impacting silk protein synthesis or the structural properties of silk fibres. Investigating the biochemical constituents of the extract and their influence on silk fibre formation could elucidate the underlying mechanisms. These findings highlight the extract's potential in enhancing filament weight, indicating its possible applications in silk quality improvement.

Table 1: Effect of *Spinacia oleracea* (1%, 2% & 3%) plant extract on biological characters of silkworm

Sr. no	Characters	Control group	1% conc.	2% conc.	3% conc.
1	Larval weight (gm)	2.650	3.401	4.084	4.501
2	Total mortality	5	3	1	0

3	Cocoon weight (gm)	1.555	1.812	2.245	2.599
4	Shell weight	0.331	0.340	0.51	0.610
5	Pupal weight (gm)	1.232	1.455	1.701	1.939
6	Shell ratio (%)	16.286	18.764	22.717	23.471
7	Filament length (M)	748	757.6	805.5	956
8	Filament weight (gm)	0.179	0.295	0.322	0.457
9	Denier	2.153	3.504	3.598	4.302
10	Number of breakages	2	0	0	0



Graph 1: Effect of *Spinacia oleracea* (1%, 2% & 3%) plant extract on biological characters of silkworm

Photo Plate 1: Rearing of Silkworm & their parameters

Conclusion

The study demonstrates that *Spinacia oleracea* extract positively influences various aspects of *Bombyx mori* L. development and silk production. Higher concentrations correlated with increased larval weights, reduced mortality, and improved cocoon parameters like weight, shell characteristics, and silk quality. These findings suggest the extract's potential to enhance sericulture practices and silk industry outcomes. Further exploration of its bioactive compounds and mechanisms is crucial for maximizing its beneficial effects in silk production.

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